This Page Is Inserted by IFW Operations
and is not a part of the Official Record

BEST AVAILABLE IMAGES

Defective images within this document are accurate representations of the original documents submitted by the applicant.

Defects in the images may include (but are not limited to):

- BLACK BORDERS
- TEXT CUT OFF AT TOP, BOTTOM OR SIDES
- FADED TEXT
- ILLEGIBLE TEXT
- SKEWED/SLANTED IMAGES
- COLORED PHOTOS
- BLACK OR VERY BLACK AND WHITE DARK PHOTOS
- GRAY SCALE DOCUMENTS

IMAGES ARE BEST AVAILABLE COPY.

As rescanning documents *will not* correct images, please do not report the images to the Image Problem Mailbox.

(12) INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(19) World Intellectual Property Organization
International Bureau

(43) International Publication Date
21 December 2000 (21.12.2000)

PCT

(10) International Publication Number
WO 00/76537 A2

(51) International Patent Classification[7]: A61K 39/00

(21) International Application Number: PCT/US00/11008

(22) International Filing Date: 24 April 2000 (24.04.2000)

(25) Filing Language: English

(26) Publication Language: English

(30) Priority Data:
09/254,556 16 June 1999 (16.06.1999) US

(63) Related by continuation (CON) or continuation-in-part (CIP) to earlier applications:
US 09/254,556 (CIP)
Filed on 16 June 1999 (16.06.1999)
US PCT/US97/15920 (CIP)
Filed on 10 September 1997 (10.09.1997)
US 09/254,556 (CIP)
Filed on 16 June 1999 (16.06.1999)

(71) Applicants *(for all designated States except US)*: **THE GOVERNMENT OF THE UNITED STATES OF AMERICA, as represented by THE SECRETARY, DEPARTMENT OF HEALTH AND HUMAN SERVICES** [US/US]; National Institutes of Health, Office of Technology Transfer, Suite 325, 6011 Executive Boulevard, Rockville, MD 20852-3804 (US). **MEDICAL UNIVERSITY OF SOUTH CAROLINA** [US/US]; 171 Ashley Avenue, Charleston, SC 29425 (US).

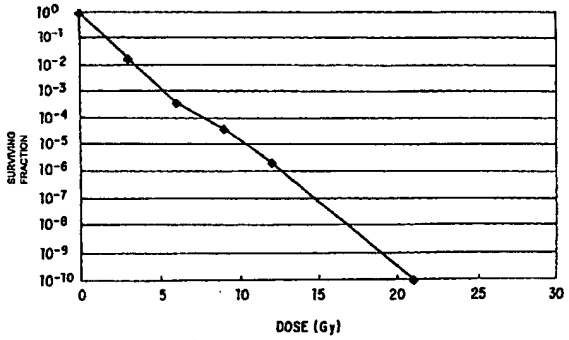
(72) Inventors; and

(75) Inventors/Applicants *(for US only)*: **GATTONI-CELLI, Sebastiano** [US/US]; 1165 Cheresonese Round, Mount Pleasant, SC 29464 (US). **SHEARER, Gene** [US/US]; 5512 Glenwood Road, Bethesda, MD 20817 (US). **GRENE, Edith** [US/US]; 9103 Friars Road, Bethesda, MD 20817 (US). **NEWTON, Danforth, A.** [US/US]; 1673 Dogwood Road, Charleston, SC 29414 (US). **BROWN, Edwin, A.** [US/US]; 773 Creekside Drive, Mount Pleasant, SC 29464 (US). **BERZOFSKY, Jay, A.** [US/US]; 5908 Bradley Boulevard, Bethesda, MD 20814-1107 (US). **DEGROOT, Anne, S.** [US/US]; 292 Morris Avenue, Providence, RI 02906 (US).

(74) Agents: **MILLER, Mary, L.** et al.; Needle & Rosenberg, P.C., Suite 1200, 127 Peachtree Street, N.E., Atlanta, GA 30303 (US).

[Continued on next page]

(54) Title: USE OF SEMI-ALLOGENEIC CELL LINE-PEPTIDE COMPLEXES FOR THE TREATMENT OF CANCER, AIDS AND OTHER VIRAL DISEASES



(57) Abstract: The present invention provides a composition comprising a semi-allogeneic hybrid fusion cell and an immunogenic peptide. In particular, isolated peptides of HIV (Human Immunodeficiency Virus), HTLV-1, Hepatitis B virus, Hepatitis C virus, rubeola virus, influenza A virus and Human Papilloma Virus are provided in the compositions of the present invention. Moreover, isolated cancer-specific peptides specific to a cancer, for example, B cell lymphoma, T cell lymphoma, myeloma, leukemia, breast cancer, pancreatic cancer, colon cancer, lung cancer, renal cancer, liver cancer, prostate cancer, melanoma and cervical cancer are provided in the compositions of the present invention. Moreover, the present invention provides a method of treating a subject infected by one or more of HIV, HTLV-1, Hepatitis B virus, Hepatitis C virus, rubeola virus, influenza A virus and Human Papilloma Virus, comprising administering a composition comprising an effective amount of a hybrid fusion cell and an effective amount of an isolated immunogenic peptide of the virus in a pharmaceutically acceptable carrier. Further, the present invention provides a method of treating cancer in a subject with one or more of B cell lymphoma, T cell lymphoma, myeloma, leukemia, breast cancer, pancreatic cancer, colon cancer, lung cancer, renal cancer, liver cancer, prostate cancer, melanoma and cervical cancer, comprising administering a composition comprising an effective amount of a hybrid fusion cell and an effective amount of an isolated immunogenic peptide of the cancer in a pharmaceutically acceptable carrier.

WO 00/76537 A2

(81) **Designated States** *(national)*: AE, AG, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, CA, CH, CN, CR, CU, CZ, DE, DK, DM, DZ, EE, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MA, MD, MG, MK, MN, MW, MX, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TR, TT, TZ, UA, UG, US, UZ, VN, YU, ZA, ZW.

(84) **Designated States** *(regional)*: ARIPO patent (GH, GM, KE, LS, MW, SD, SL, SZ, TZ, UG, ZW), Eurasian patent (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European patent (AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE), OAPI patent (BF, BJ, CF, CG, CI, CM, GA, GN, GW, ML, MR, NE, SN, TD, TG).

Published:

— *Without international search report and to be republished upon receipt of that report.*

For two-letter codes and other abbreviations, refer to the "Guidance Notes on Codes and Abbreviations" appearing at the beginning of each regular issue of the PCT Gazette.

USE OF SEMI-ALLOGENEIC CELL LINE-PEPTIDE COMPLEXES FOR THE TREATMENT OF CANCER, AIDS AND OTHER VIRAL DISEASES

BACKGROUND OF THE INVENTION

FIELD OF THE INVENTION

The present invention relates to the field of treatment of cancer, Acquired Immune Deficiency Syndrome (AIDS) and other viral diseases. In particular, the present invention provides a method of immunotherapy for the treatment of cancer, AIDS and other viral diseases.

BACKGROUND ART

The possibility has been raised that alloimmunization can serve to supplement deficient T cell help in HIV^+ patients (27). Because of the recent increased incidence in failure of highly active anti-retroviral therapy (HAART) in AIDS patients, protocols are needed to complement HAART with immune-based therapy (IBT) (28). Alloimmunization would represent a unique IBT strategic approach because: 1) PBMC from a higher percentage of HIV^+ patients generated *in vitro* Th responses to allogeneic PBMC than to recall antigens (29); 2) Th cell defects for generating influenza A virus (FLU)-specific CTL in AIDS patients was circumvented by *in vitro* costimulation of patients' PBMC with FLU plus allogeneic PBMC (30); and 3) APC from uninfected allogeneic donors can provide a source of APC with intact antigen-presenting molecules and function that are deficient in some HIV^+ patients (31). However, costimulation of AIDS patients' PBMC with allogeneic cells and HIV antigens to generate HIV-specific cellular immunity has not been investigated.

Allo-specific help might be most effective in the patient if the same APC that presents the HIV peptide to the T effector cell (Te) also presents MHC alloantigens to the alloantigen-specific Th cell (27). An APC presenting both

helper and effector antigens might provide a better chance for Th cytokines to enhance the HIV-specific Te function, because these two interacting T cells would be necessarily activated by a common APC in the same microenvironment. This strategy could provide optimal interaction among Th, APC and CTL (three-cell model) (27, 32-34). To address the potential three-cell problem in AIDS in which virus-specific Th may be selectively deficient, we prepared semi-allogeneic cell hybrids from the fusion of HIV^+ patient PBMC with the allogeneic cell line FO1-12 (35). Cell hybrids prepared by fusion techniques have been used previously in murine tumor immunology (11-13). Irradiated, semi-allogeneic cell hybrids engineered for individual AIDS patients could permit efficient co-recognition of HLA allogeneic as well as MHC self-restricted viral antigenic determinants on the same APC, and might complement HAART therapy for decreasing viral load, increasing $CD4^+$ counts, and restoring CTL function to HIV and other pathogenic viruses.

The present study demonstrates that : 1) semi-allogeneic hybrids can be produced by fusion of FO1-12 with HIV^+ patients' PBMC; 2) these hybrids express HLA class I and II antigens from both fusion partners, as well as the CD86 costimulatory molecule, and stimulate T cell proliferation; 3) the hybrids can present HIV envelope peptides (*env*) to patients' T cells, resulting in the generation of *env*-specific CTL responses; and 4) costimulation of patients' T cells with *env*-pulsed semi-allogeneic hybrids results in enhanced *env*-specific CTL activity compared to stimulation with *env* alone.

SUMMARY OF THE INVENTION

The present invention provides a composition comprising a semi-allogeneic hybrid fusion cell and an immunogenic peptide.

Further provided is a composition comprising isolated *env* T1, isolated *env* T2, isolated *env* Th4.1, isolated *env* P18 and isolated *env* P18 MN.

A composition comprising isolated nef, isolated gag and isolated Tat is also provided.

Also provided is a composition comprising isolated *env* T1, isolated *env* T2, isolated *env* Th4.1, isolated *env* P18, isolated *env* P18 MN, isolated nef, isolated gag and isolated Tat.

Further provided in the present invention is a composition comprising at least two isolated peptides selected from the group consisting of isolated *env* T1, isolated *env* T2, isolated *env* Th4.1, isolated *env* P18, isolated *env* P18 MN, isolated nef, isolated gag and isolated Tat.

The present invention provides a method of treating an HIV infection in a subject, comprising administering to the subject an effective amount of an immunogenic HIV-specific peptide in a pharmaceutically acceptable carrier and an effective amount of a hybrid fusion cell in a pharmaceutically acceptable carrier, wherein the hybrid fusion cell is a fusion of a cell that is deficient in β_2 microglobulin, resistant to neomycin and sensitive to HAT and a white blood cell from the subject.

The present invention provides a composition comprising isolated immunogenic peptides of HTLV-1, Hepatitis B virus, Hepatitis C virus, rubeola virus, influenza A virus and Human Papilloma Virus.

Further provided by the present invention is a composition comprising a semi-allogeneic hybrid fusion cell and an immunogenic peptide of a virus selected from the group consisting of HTLV-1, Hepatitis B virus, Hepatitis C virus, rubeola virus, influenza A virus and Human Papilloma Virus.

Also provided is a method of treating a subject infected with at least one virus selected from the group consisting of HTLV-1, Hepatitis B virus, Hepatitis C virus, rubeola virus and influenza A virus, comprising administering to the subject an effective amount of an isolated virus-specific immunogenic peptide selected from the group consisting of a peptide of HTLV-1, a peptide of Hepatitis B virus, a peptide of Hepatitis C virus, a peptide of rubeola virus, a peptide of influenza A virus and a peptide of Human Papilloma Virus in a pharmaceutically acceptable carrier and an effective amount of a hybrid fusion cell in a pharmaceutically acceptable carrier, wherein the hybrid fusion cell is a fusion of a cell that is deficient in β_2 microglobulin, resistant to neomycin and sensitive to HAT and a white blood cell derived from the subject infected by a virus.

The present invention further provides a composition comprising cancer-specific immunogenic peptides selected from the group consisting of B cell lymphoma, T cell lymphoma, myeloma, leukemia, breast cancer, pancreatic cancer, colon cancer, lung cancer, renal cancer, liver cancer, prostate cancer, melanoma and cervical cancer in a pharmaceutically acceptable carrier.

The present invention also provides a method of treating cancer in a subject, comprising administering to the subject an effective amount of an cancer-specific immunogenic peptide in a pharmaceutically acceptable carrier and an effective amount of a hybrid fusion cell in a pharmaceutically acceptable carrier, wherein the hybrid fusion cell is a fusion of a cell that is deficient in β_2 microglobulin, resistant to neomycin and sensitive to HAT and a white blood cell from the subject.

BRIEF DESCRIPTION OF THE DRAWINGS

Fig. 1 shows the survival curve of FO-1-neo cells exposed to increasing doses of γ-rays.

Fig. 2 shows the surface expression of HLA class I antigens on parental cells (FO-1 #12 and 501) and on tumor cell hybrids (FO-1 #12 X 501) obtained from their fusion. Single-cell suspensions from each culture (indicated at the side of the figure) were subjected to indirect immunofluorescent staining and reacted with second antibody alone (blank), monoclonal antibody (mAb) W6-32 (anti-HLA-A,B,C + β_2 microglobulin), and mAb PA2.1 (anti-HLA-A2). Fluorescence intensity was determined by flow cytometry on a Becton-Dickinson cell analyzer. Note that FO-1 clone 12 (FO-1) cells do not express HLA class I antigens because they lack β_2 microglobulin expression; in contrast, tumor cell hybrids (FO-1 #12 X 501) grown in selective medium containing HAT and the neomycin analog G418 (600 µg/ml) express on the cell surface HLA class I antigens, including HLA-A2 (this antigen derives from 501 parental cells).

Fig. 3 shows the survival curve of 2 melanoma hybrids (FO-1 #12 x WM2; FO-1 #12 x JJ) exposed to γ-rays.

Fig. 4 shows proliferative responses of PBMC from HIV^+ patients stimulated with semi-allogeneic hybrids and with the allogeneic FO1-12 line. PBMC from three HIV^+patients (#6, #3 and #1) were stimulated for 5 days with irradiated (25 Gy) donor-derived semi-allogeneic hybrids or the allogeneic line FO1-12 or *env* peptide pool.

Fig. 5 shows IL-2 production and *env*-specific CTL generation comparing semi-allogeneic hybrids that were irradiated (irrad) with those that were treated with mitomycin C (MMC). (A): IL-2 production by untreated hybrid , irrad hybrid , or MMC treated. (B): generation of *env*-specific CTL by co-stimulation with *env* peptides plus hybrids that were irradiated (○) or treated with MMC (●) before co-culturing with PBMC and *env* peptides.

Fig. 6 shows CTL activity generated by donor PBMC stimulated with semi-allogeneic hybrids (PBMC+hyb), PBMC stimulated with hybrids plus *env*

peptide pool (PBMC+hyb+env) or *env* pool alone (PBMC+env). The CTL effectors generated were assayed on autologous EBV-transformed targets pulsed with *env* peptide pool (upper panels) or with media alone (lower panels). Patients #1, #3, #4, and #6 represent the responses observed by PBMC from 4/6 HIV^+ patients tested. Patients #3, #4, and #6 illustrate the data from the three CTL responders; patient #1 shows negative data representative of the three CTL non-responders.

Fig. 7 shows PBMC from patients #3 (that contain both T cells and autologous APC) were stimulated: with envelope peptides (*env*) in culture for 6 days (●); by the pulsing PBMC with *env* for 1 hr, followed by washing out *env* and 6 days in culture (○); with *env* plus hybrid cells (hyb) for 6 days (▲); by pulsing the PBMC with *env* for 1 hr, followed by washing out *env* and adding hyb for 6 days (❒); by pulsing hyb cells with *env* for 1 hr, followed by washing out *env* and adding PBMC for 6 days (■); or by co-culture of PBMC with hyb alone (negative control) (◆).

Fig. 8 shows models illustrating the interaction between: (A) semi-allogeneic (hybrid) APC that stimulate allo-specific T helper cells via allo-MHC (class II) and autologous APC expressing *env*+self-MHC (class I) that stimulate *env*-specific CTL precursors ; and (B) semi-allogeneic hybrid APC that concomitantly stimulate allo-specific T helper cells via allo MHC (class II) and *env*-specific CTL precursors via *env*+self-MHC (class I).

Fig. 9 shows CTL activity of donor PBMC stimulated with semi-allogeneic hybrids (RC+semiallo), hybrids plus *env* peptide pool (RC+semiallo+env), or *env* pool alone (RC+env). The CTL effectors generated were assayed on autologous EBV-transformed targets pulsed with *env* peptide pool (upper panels) or with media alone (lower panels). Patients #1 (panel B), #3 (panel A), #4 (panel D), and #6 (panel C)

represent the responses observed by PBMC from four of six HIV^+ patients tested. Patients #3 (panel A), #4 (panel D), and #6 (panel C) illustrate the data from the three CTL responses; patient #1 (panel B) shows negative data representative of the three CTL non-responders.

Fig. 10 shows CTL activity by a patient's (#3) PBMC stimulated by: pulsing the PBMC with *env* peptides for 1 hr before six day culture (RC+env*); PBMC in culture with *env* for six days (RC+env); PBMC in culture with *env* and hybrids for six days (RC+env+hyb); pulsing of PBMC with *env* before adding hybrid cells for six days (RC+env*+hyb); pulsing of hybrid cells with *env* before adding PBMC for six days (RC+hyb+env*); PBMC in culture for six days with hybrid cells (RC+hyb).

Fig. 11 shows results of cytotoxicity assays (^{51}Cr release) showing % lysis values of T2 target cells loaded with HLA-A2-restricted exogenous peptides (FLU-M1 or HIV-1-derived GAG). Unloaded T2 cells and Daudi cells were used as controls. Each panel (A-F) represents PBMC effector cells from an HIV-infected, $HLA\text{-}A2^+$ patient that have been exposed to: A) no antigen; B) irradiated, semi-allogeneic hybrids (HYB); C) FLU-M1 peptide; D) FLU-M1 peptide plus irradiated, semi-allogeneic hybrids; E) GAG peptide; or F) GAG peptide plus irradiated, semi-allogeneic hybrids. Lysis with effector:target ratios of 1:1 and 5:1 are shown.

DETAILED DESCRIPTION OF THE INVENTION

As used herein, "a," "an" or "the" may mean one or more. For example, "a" cell may mean one cell or more than one cell. Further, "the" cell may mean one or more than one cell.

Hybrid Fusion Cell and Immunogenic Peptide Combination

The present invention provides a composition comprising a semi-allogeneic hybrid fusion cell and an immunogenic peptide. The immunogenic peptide, for

example, can be a virus-specific peptide or a cancer-specific peptide specific for a cancer.

As described below, the hybrid fusion cell is a fusion cell that is deficient in β_2 microglobulin, resistant to neomycin and sensitive to HAT and a cell of a human subject. Typically the cell originates from one subject and is used to produce the hybrid fusion cell of the present invention for the treatment of the same subject diagnosed with cancer, AIDS or other viral diseases. The cell can be, for example, a peripheral blood mononuclear cell (PBMC), such as a lymphocyte or a monocyte. Moreover, the PBMC is usually a cell that is not infected by a virus. Moreover, the cell can come from a subject who does not have cancer, AIDS or other viral diseases for future use in a hybrid fusion cell.

The present invention provides a composition comprising a semi-allogeneic hybrid fusion cell and an isolated HIV-specific peptide. The hybrid fusion cell is a fusion of the FO-1 #12 cell, described below, and a PBMC derived from an HIV-positive subject.

The present invention provides a composition wherein the hybrid fusion cell presents a virus-specific immunogenic peptide. By "presents" is meant that the hybrid fusion cell acts like an antigen presenting cell. The hybrid fusion cell ingests a foreign antigen and/or displays the foreign antigen bound to a class II MHC protein on its surface. The subject's helper T cells bind to the foreign antigen bound to a class II MHC protein and become activated, subsequently activating cytotoxic T cells by secreting cytokines, such as interleukins.

The present invention provides a composition wherein the hybrid fusion cell presents an isolated immunogenic HIV-specific peptide. "Isolated" as used herein means the peptide of this invention is sufficiently free of contaminants or cell components with which peptides normally occur and is present in such concentration as to be the only significant peptide present in the sample. "Isolated" does not mean that

the preparation is technically pure (homogeneous), but it is sufficiently pure to provide the peptide in a form in which it can be used therapeutically. In the present invention, an isolated HIV-specific peptide is free of other peptides and nucleic acids comprising HIV. As used herein, "immunogenic" means able to produce an immune response in a subject.

An HIV-specific peptide can be an envelope peptide or a core peptide. Examples of isolated HIV-specific envelope peptides include, but are not limited to, isolated *env* T1, isolated *env* T2, isolated *env* Th4.1, isolated *env* P18 and isolated *env* P18 MN. Moreover, examples of isolated HIV-specific core peptides include, but are not limited to, isolated nef, isolated gag and isolated Tat.

The present invention thus provides a composition comprising the hybrid fusion cell and an isolated HIV-specific envelope peptide, wherein the envelope peptide is selected from the group consisting of isolated *env* T1, isolated *env* T2, isolated *env* Th4.1, isolated *env* P18 and isolated *env* P18 MN. Moreover, the present invention provides a composition comprising the hybrid fusion cell and at least two isolated peptides selected from the group consisting of isolated *env* T1, isolated *env* T2, isolated *env* Th4.1, isolated *env* P18 and isolated *env* P18 MN.

Moreover, the present invention provides a composition comprising isolated *env* T1, isolated *env* T2, isolated *env* Th4.1, isolated *env* P18 and isolated *env* P18 MN.

Further, the present invention provides a composition comprising the hybrid fusion cell of the invention and at least two isolated peptides selected from the group consisting of nef, gag and Tat.

The present invention further provides a composition comprising isolated nef, isolated gag and isolated Tat. Moreover, the present invention provides a composition comprising isolated *env* T1, isolated *env* T2, isolated *env* Th4.1, isolated *env* P18, isolated *env* P18 MN, isolated nef, isolated gag and isolated Tat.

The present invention also provides a composition comprising at least two isolated peptides selected from the group consisting of isolated *env* T1, isolated *env* T2, isolated *env* Th4.1, isolated *env* P18, isolated *env* P18 MN, isolated nef, isolated gag and isolated Tat.

Moreover, the present invention provides a composition comprising a semi-allogeneic hybrid fusion cell and an isolated immunogenic peptide of a virus selected from the group consisting of HTLV-1, Hepatitis B virus, Hepatitis C virus, rubeola virus, influenza A virus and Human Papilloma Virus. A peptide can be isolated from the wild virus. Moreover, a peptide can be produced when the sequence of the nucleic acid encoding the amino acid sequence of the peptide is known. Thus, a person of skill in the art can express the product of the nucleic acid sequence by genetic engineering methods known in the art. Further, a person of skill in the art can synthesize an isolated peptide of a virus by chemical means when the amino acid sequence comprising the peptide is known.

The present invention provides a composition wherein the hybrid fusion cell is a fusion of a cell that is deficient in β_2 microglobulin, resistant to neomycin and sensitive to HAT and a white blood cell derived from a subject infected with a virus selected from the group consisting of HTLV-1, Hepatitis B virus, Hepatitis C virus, rubeola virus, influenza A virus and Human Papilloma Virus. The hybrid fusion cell is the cell as described below.

The present invention provides a composition wherein the hybrid fusion cell presents a virus-specific immunogenic peptide. As noted above, the hybrid fusion cell acts as an antigen-presenting cell. Examples of virus-specific immunogenic peptides include, but are not limited to, those peptides of HTLV-1, Hepatitis B virus, Hepatitis C virus, rubeola virus, influenza A virus and Human Papilloma Virus.

The present invention further provides a composition comprising a hybrid fusion cell and a virus-specific immunogenic peptide, wherein the virus-specific

immunogenic peptide is selected from the group consisting of an isolated peptide of HTLV-1, an isolated peptide of Hepatitis B virus, an isolated peptide of Hepatitis C virus, an isolated peptide of rubeola virus, an isolated peptide of influenza A virus and an isolated peptide of Human Papilloma Virus.

Moreover, the present invention provides a composition comprising a fusion hybrid cell and at least two isolated peptides selected from the group consisting of a peptide of HTLV-1, a peptide of Hepatitis B virus, a peptide of Hepatitis C virus, a peptide of rubeola virus, a peptide of influenza A virus and a peptide of Human Papilloma Virus.

The present invention further provides a composition comprising isolated peptides of HTLV-1, Hepatitis B virus, Hepatitis C virus, rubeola virus, influenza A virus and Human Papilloma Virus in a pharmaceutically acceptable carrier.

Further, the present invention provides a composition comprising a population of PBMCs enriched for cytotoxic T cells produced by the method of the invention. The cytotoxic T cells are directed toward the immunogenic peptides that the hybrid fusion cell presents.

Thus, a composition comprising a population of peripheral blood mononuclear cells enriched for cytotoxic T cells produced by the method as described below is provided. The composition can be in a pharmaceutically acceptable carrier.

The present invention further provides a composition comprising a semi-allogeneic hybrid fusion cell and an immunogenic cancer-specific peptide. The cancer-specific immunogenic peptide can be from any cancer including, but not limited to, B cell lymphoma, T cell lymphoma, myeloma, leukemia, breast cancer, pancreatic cancer, colon cancer, lung cancer, renal cancer, liver cancer, prostate cancer, melanoma and cervical cancer. Examples of immunogenic cancer-specific peptides include any tumor antigen now known or later identified as a tumor antigen. For example, the tumor

antigen can be, but is not limited to, human epithelial cell mucin (Muc-1; a 20 amino acid core repeat for Muc-1 glycoprotein, present on breast cancer cells and pancreatic cancer cells), the Ha-ras oncogene product, p53, carcino-embryonic antigen (CEA), the raf oncogene product, GD2, GD3, GM2, TF, sTn, MAGE-1, MAGE-3, tyrosinase, gp75, Melan-A/Mart-1, gp100, HER2/neu, EBV-LMP 1 & 2, HPV-F4, 6, 7, prostatic serum antigen (PSA), alpha-fetoprotein (AFP), CO17-1A, GA733, gp72, p53, the ras oncogene product, HPV E7 and melanoma gangliosides, as well as any other tumor antigens now known or identified in the future. Tumor antigens can be obtained following known procedures or are commercially available. (64)

Moreover, the present invention provides a composition comprising cancer-specific immunogenic peptides selected from the group consisting of B cell lymphoma, T cell lymphoma, myeloma, leukemia, breast cancer, pancreatic cancer, colon cancer, lung cancer, renal cancer, liver cancer, prostate cancer, melanoma and cervical cancer in a pharmaceutically acceptable carrier.

Thus, the present invention provides a population of PBMCs enriched for cytotoxic T cells produced by the method of the present invention. The PBMCs can be in a pharmaceutically acceptable carrier.

Treating Disease Using the Hybrid Fusion Cell-Peptide Combination

The present invention provides a method of enhancing the cytotoxic T cell activity of a population of peripheral blood mononuclear cells of a human subject, comprising contacting the peripheral blood mononuclear cells (PBMCs) of the subject with a composition comprising a hybrid fusion cell and an immunogenic peptide. For example, the immunogenic peptide can be a virus-specific peptide, such as an HIV-specific peptide. Examples of HIV-specific peptides include, but are not limited to, isolated *env* T1, isolated *env* T2, isolated *env* Th4.1, isolated *env* P18, isolated *env* P18 MN, isolated nef, isolated gag and isolated Tat.

The contacting step can occur *in vitro* or *in vivo*. When the contacting step occurs *in vitro*, sufficient time is allowed to increase the numbers and cytotoxic activity of CTL in the population. The amount of time may vary but is expected to be in the range of one to two weeks. An example of this method is provided in the Examples herein. When the contacting step occurs *in vivo*, it follows the concurrent administration of the hybrid fusion cell and peptide combination as described in the Examples herein.

Moreover, the present invention provides a method of treating a viral infection in a subject, comprising administering to the subject an effective amount of a composition of the present invention comprising a population of PBMCs enriched for cytotoxic T cells produced as described below, in a pharmaceutically acceptable carrier. As noted above, an example of a viral infection that can be treated according to the method of the present invention is HIV infection.

The present invention further provides a method of treating an HIV infection in a subject, comprising administering to the subject an effective amount of the hybrid fusion cell of the present invention and an effective amount of at least one isolated immunogenic HIV-specific peptide in a pharmaceutically acceptable carrier. In general, an " effective amount" of an agent is that amount needed to achieve the desired result or results. Thus, a therapeutically effective amount of the composition of the present invention can decrease viral load, increase $CD4^+$ counts and restore CTL function to counter the HIV. Moreover, by "pharmaceutically acceptable" is meant a material that is not biologically or otherwise undesirable, i.e., the material may be administered to an individual along with the selected peptide or cell without causing substantial deleterious biological effects or interacting in a deleterious manner with any of the other components of the composition in which it is contained. Furthermore, any of the compositions of this invention can comprise a pharmaceutically acceptable carrier.

The present invention provides a method of treating an HIV infection in a subject, comprising administering to the subject an effective amount of an HIV-specific peptide in a pharmaceutically acceptable carrier and an effective amount of a hybrid fusion cell in a pharmaceutically acceptable carrier, wherein the hybrid fusion cell is a fusion of a cell that is deficient in β_2 microglobulin, resistant to neomycin and sensitive to HAT and a white blood cell derived from the subject. The hybrid fusion cell and the HIV-specific peptide are administered concurrently as described in the Examples herein. At least one of the following isolated HIV-specific peptides is a component of the composition which is administered to the subject: *env* T1, *env* T2, *env* Th4.1, *env* P18 and *env* P18 MN, nef, gag and Tat.

The present invention provides a method enhancing the cytotoxic T cell activity of a population of peripheral blood mononuclear cells of a subject infected with at least one virus selected from the group consisting of HTLV-1, Hepatitis B virus, Hepatitis C virus, rubeola virus, influenza A virus and Human Papilloma Virus, comprising contacting the peripheral blood mononuclear cells with a hybrid fusion cell of the present invention and an immunogenic peptide of a virus selected from the group consisting of HTLV-1, Hepatitis B virus, Hepatitis C virus, rubeola virus, influenza A virus and Human Papilloma Virus. The contacting can occur *in vitro* or *in vivo*.

The present invention provides a method of treating a subject infected with a least one virus selected from the group consisting of HTLV-1, Hepatitis B virus, Hepatitis C virus, rubeola virus, influenza A virus and Human Papilloma Virus, comprising administering to the subject an effective amount of the composition comprising a population of PBMCs enriched for cytotoxic T cells in a pharmaceutically acceptable carrier. The cytotoxic T cells are directed toward the immunogenic peptide of the virus that infects the subject.

Also provided is a method of treating a subject infected with at least one virus selected from the group consisting of HTLV-1, Hepatitis B virus, Hepatitis C virus, rubeola virus, influenza A virus and Human Papilloma Virus, comprising administering

to the subject an effective amount of a composition comprising a semi-allogeneic hybrid fusion cell and an isolated peptide of a virus selected from the group consisting of HTLV-1, Hepatitis B virus, Hepatitis C virus, rubeola virus, influenza A virus and Human Papilloma Virus in a pharmaceutically acceptable carrier.

Further provided is a method of treating a subject infected with at least one virus selected from the group consisting of HTLV-1, Hepatitis B virus, Hepatitis C virus, rubeola virus, influenza A virus and Human Papilloma Virus, comprising administering to the subject an effective amount of an isolated immunogenic virus-specific peptide selected from the group consisting of a peptide of HTLV-1, a peptide of Hepatitis B virus, a peptide of Hepatitis C virus, a peptide of rubeola virus, a peptide of influenza A virus and a peptide of Human Papilloma Virus in a pharmaceutically acceptable carrier and an effective amount of a hybrid fusion cell in a pharmaceutically acceptable carrier wherein the hybrid fusion cell is a fusion of a cell that is deficient in β_2 microglobulin, resistant to neomycin and sensitive to HAT and a white blood cell of the subject infected by a virus. The dosage regimen for this method of treatment is similar to that of the method for treating a subject infected with HIV, as taught above and described in the Examples herein. The hybrid fusion cell and isolated immunogenic virus-specific peptide are administered concurrently.

Further, the present invention provides a method of enhancing the cytotoxic T cell activity of a population of peripheral blood mononuclear cells of a human subject, comprising contacting the peripheral blood mononuclear cells of the subject with the hybrid fusion cell of the present invention and an immunogenic cancer-specific peptide. The contacting can be *in vitro* or *in vivo*. The cytotoxic T cells that are enhanced in activity are directed toward an immunogenic cancer-specific peptide.

Moreover, the present invention provides a method of treating cancer in a human subject, comprising administering to the subject an effective amount of a composition of cytotoxic T cells with enhanced activity directed toward an immunogenic cancer-specific peptide in a pharmaceutically acceptable carrier.

Moreover, the present invention provides a method of treating cancer in a subject, comprising administering to the subject an effective amount of a cancer-specific immunogenic peptide in a pharmaceutically acceptable carrier and an effective amount of a hybrid fusion cell in a pharmaceutically acceptable carrier, wherein the hybrid fusion cell is a fusion of a cell that is deficient in β_2 microglobulin, resistant to neomycin and sensitive to HAT and a white blood cell from the subject. The immunogenic cancer-specific peptide and the hybrid fusion cell can be administered to the subject concurrently.

The compositions of the present invention can be administered to a subject by parenteral routes of administration which include, but are not limited to, intravenously, intramuscularly, intradermally, subcutaneously, intraperitoneally and intrathecally.

"Allo" Cell.

The invention provides "allo" cells capable of fusing with patient-derived cells to form semi-allogeneic cell hybrids. A "patient-derived" cell is a cell from a subject who will receive fusion cells of this invention. The allo cell provided herein is the fusion partner of a patient ("self") cell in the present semiallogeneic cell hybrids. The allo cell of the present invention is an isolated cell or cell line, wherein the cell is deficient in β_2 microglobulin, has a selectable dominant marker and a selectable recessive marker. The cell or cell line is preferably human, or human-derived. An example of a cell having these characteristics is the cell line designated FO-1 #12.

The allo cell or cell line as described, wherein the dominant marker, drug or antibiotic resistance, is provided. The antibiotic resistance can be, for example, to neomycin or to any other drug or antibiotic resistance marker as is well known in the art. There are numerous examples in the art of expression of a selectable dominant marker associated with resistance to drug/antibiotic other than neomycin: hygromycin, methotrexate, α-amanitin, ouabain, etc.

The allo cell or cell line as described, wherein the recessive marker is sensitivity to aminopterin-containing medium (sensitivity to hypoxanthine + aminopterin + thymidine (HAT)-containing medium) is provided. There are other examples in the art of recessive selectable markers, such as deficiency in thymidine kinase.

The cell FO-1 #12 is characterized as being β_2 microglobulin deficient, neomycin-resistant and HAT-sensitive. An example of a method for making such a cell is given in the Examples herein. A cell having the characteristics of the cell line designated FO-1 #12 and deposited on August 27, 1996 with the American Type Culture Collection, 12301 Parklawn Drive, Rockville, MD 20852 under accession number ATCC CRL-12177 is also provided.

Disease-Specific Antigen-Expressing Allo Cells.

In an alternative embodiment, an allo cell of the invention is provided, wherein the cell expresses a heterologous antigen. The terms "antigen" and "antigen fragment" and "antigenic" as used herein, mean a protein (peptide, polypeptide, etc.) capable of inducing an immunogenic T cell-mediated response. The antigen, when expressed, can be presented at least in part on the surface of a cell, bound to an HLA class I molecule. Thus, the heterologous antigen need not be thought of as a typical cell surface antigen. For example, prostate-specific antigen (PSA) is a cytoplasmic protein, but gives rise to cellular immunity, because fragments of it are presented on the cell surface of cancer cells, bound to HLA class I molecules.

The allo cell can express a heterologous nucleic acid encoding an antigen that is a tumor antigen. The heterologous nucleic acid is introduced into the allo cell by methods well known in the art, such as transfection or transduction. The tumor antigen of this invention can be gp100/pmel17 (which is constitutively expressed by FO-1 #12), carcino-embryonic antigen (CEA), MUC-1, HER-2/neu, MAGE-1, MAGE-3, BAGE, GAGE, tyrosinase, MART-1, gp75, MUM, HPV-16, prostate-specific antigen (PSA), and other breast cancer-specific antigens, colon cancer-specific antigens, lung cancer-

specific antigens, pancreatic cancer-specific antigens, prostate cancer-specific antigens, HPV-specific antigens (23), or other antigens.

The allo cell can express a virus-specific antigen. For example the cell can express an HIV-specific antigen. The HIV-specific antigen can, for example, be gag or an antigenic fragment thereof, pol or an antigenic fragment thereof, env or an antigenic fragment thereof or nef or an antigenic fragment of it (24, 25).

A cell expressing a polypeptide fragment of the heterologous antigen is also provided. This cell can be the allo cell that will be fused to the patient cell. The term "fragment" as used herein regarding antigens, means a molecule of at least 5 contiguous amino acids that has an antigenic function as described herein. As used herein to describe an amino acid sequence (protein, polypeptide, peptide, etc.), "specific" means that the amino acid sequence is not found identically in any other source. Thus, for example, "virus-specific" (e.g., HIV-specific) means that the amino acid sequence is found only in the HIV virus, and not found identically in any other source. The determination of specificity is made routine, because of the availability of computerized amino acid sequence databases, wherein an amino acid sequence of almost any length can be quickly and reliably checked for the existence of identical sequences. If an identical sequence is not found, the amino acid sequence is "specific" for the recited source.

An antigenic fragment can be selected by applying the routine technique of epitope mapping to the larger antigen to determine the regions of the proteins that contain epitopes that are capable of eliciting an immune response in an animal. Once the epitope is selected, an antigenic polypeptide containing the epitope can be synthesized directly, or produced recombinantly by cloning nucleic acids encoding the polypeptide in an expression system, according to the standard methods. Alternatively, an antigenic fragment of the antigen can be isolated from the whole antigen or a larger fragment by chemical or mechanical disruption. Fragments can also be randomly chosen from a known antigen sequence and synthesized. The purified fragments thus

obtained can be tested to determine their antigenicity and specificity by routine methods or by the TIL/PBMC education methods described herein.

The heterologous antigenic polypeptides to be expressed in the present cells can be tested to determine their immunogenicity and specificity. Briefly, B lymphocytes and T cells are isolated from a patient who has an immune response to the present vaccine. The peptides expressed by the vaccine are stripped off of the vaccine cells and loaded onto B cells. Patient T cells are then tested for their ability to kill the B cells loaded with the vaccine peptides. If the T cells kill the B cells, the peptide antigen(s) eliciting the response are purified and sequenced. By identifying the peptide, synthetic vaccines can be generated.

A nucleic acid encoding a particular antigen of interest, or a region of that nucleic acid, can be constructed, modified, or isolated. That nucleic acid can then be cloned into an appropriate vector, which can direct the expression of the antigen in the allo cell. The vector is contemplated to have the necessary functional elements that direct and regulate transcription of the inserted gene, or hybrid gene. These functional elements include, but are not limited to, a promoter, regions upstream or downstream of the promoter, such as enhancers that may regulate the transcriptional activity of the promoter, an origin of replication, appropriate restriction sites to facilitate cloning of inserts adjacent to the promoter, antibiotic resistance genes or other markers which can serve to select for cells containing the vector or the vector containing the insert, RNA splice junctions, a transcription termination region, or any other region which may serve to facilitate the expression of the inserted gene or hybrid gene. (See generally, Sambrook *et al.* (*Molecular Cloning: A Laboratory Manual*, 2nd Ed., Cold Spring Harbor Laboratory, Cold Spring Harbor, New York, 1989)). The vector can be delivered to the cell for expressing the antigen-encoding nucleic acid using commercially available systems as further described below and in the literature.

Cell Hybrids.

A cell hybrid formed by the fusion between the allo cell (e.g., an FO-1 #12 cell or other cell described herein) and a mammalian cell is provided. The fusion can take place under any conditions suitable for such fusions. One set of conditions under which cell fusion take place is described in the Examples herein. It is recognized, however, that other conditions are known or can be derived that permit fusion, and this does not change the nature of the resulting hybrid.

The mammalian cell can be a cell of a human subject. In one embodiment of the hybrid, the human cell can be a white blood cell. The white blood cell can be a lymphocyte or a monocyte. In another embodiment, the cell of a human subject can be a tumor cell. The tumor cell can be a melanoma cell, a prostatic carcinoma cell, a colon carcinoma cell, a lung carcinoma cell, a breast carcinoma cell, a pancreatic carcinoma cell, prostatic carcinoma etc.

Also provided is a fused cell hybrid of the heterologous antigen-expressing cell (allo-antigen cell) of the invention and a mammalian cell. A cell hybrid, wherein the mammalian cell is a patient-derived human cell is provided. The patient-derived human cell can be a white blood cell, more conveniently a peripheral white blood cell. As used herein, "patient-derived" means of or originating from a patient.

In the fused cell hybrid of the invention, the heterologous antigen-expressing cell can express a tumor antigen. Alternatively the heterologous antigen-expressing cell can express an HIV- or HPV-specific antigen.

Thus, the semi-allogeneic vaccine of this invention comprises three components: 1) a "self" component represented by the patient-derived (-specific) HLA haplotype; 2) an "allo" component represented by any human cell line which has a different HLA haplotype; and 3) an "antigen" component which is disease-specific and may or may not be patient-derived. In one embodiment, the antigen is an immunogenic virus-specific peptide or cancer-specific peptide specific for a cancer. The

immunogenic peptide can be from a virus or cancer cell or synthesized by methods known to persons of skill in the art. Alternatively, the allo and antigen components are engineered into an appropriately modified human cell line (e.g., a cell having the characteristics of FO-1 #12) which is fused with the patient-derived self component in order to generate patient-tailored semi-allogeneic cell hybrids. It is both practical and convenient, but not necessary, to use peripheral white blood cells as the self component, since blood-drawing is a minimally invasive and rather innocuous procedure. An appropriate isolated peptide or engineered antigen component can be virus- or cancer-specific, for example, HIV-derived gag protein product (peptide or polypeptide) for preventive, as well as therapeutic AIDS vaccines; carcino-embryonic antigen (CEA) for preventive as well as therapeutic vaccination against many forms of carcinoma (colon, breast, lung, pancreatic, etc.); gp 100 for preventive as well as therapeutic vaccination against melanoma; and prostate-specific antigen (PSA) for preventive as well as therapeutic vaccination against prostatic cancer. The genetic engineering involved in producing the engineered allo/antigen cell of the hybrid is routine and can be accomplished using commercially available vectors and other reagents. The method of fusing the allo/antigen cell and the self cell to form the hybrid is also routine and described herein.

The cell hybrid provided herein can be lethally irradiated for use as a preventive or therapeutic vaccine for cancer or AIDS. The irradiation step takes place shortly before administration of the hybrid to a patient as further described in the Examples herein. Thus, an irradiated semiallogeneic cell hybrid is provided.

A method for making a cell hybrid of this invention is provided. The method includes the steps of a) contacting a cell deficient in β_2 microglobulin, having a selectable dominant marker and having a selectable recessive marker with a patient-derived tumor cell or white blood cell under conditions in which cell hybrids are formed; and b) selecting cell hybrids by determining the presence of the dominant marker and the presence of the recessive marker, whereby the presence of both the dominant and recessive markers is correlated with the presence of a cell hybrid. This

method can further comprise the step of identifying cells that express HLA class I surface antigens. An example of this method is described in detail in the Examples section herein.

Semi-allogeneic cell hybrids as preventive and therapeutic vaccines for cancer and AIDS.

A method of treating a cancer, HIV infection or other viral disease in a subject, comprising administering to the subject an effective amount of a cell hybrid of the present invention, wherein patient-derived tumor cell or white blood cell is derived from the patient being treated, is provided. By "treating" is meant an improvement in the patient's condition. The improvement can be in any of the parameters typically used by clinicians to assess the condition of the patient. For example, reduction in or stabilization of tumor mass or in antigen level in serum are evidence of efficacious treatment of a solid tumor. In the case of HIV infection or AIDS, reduction in HIV titre or increase in $CD4^+$ counts in the peripheral blood are evidence of efficacious treatment of HIV infection or AIDS.

A method of treating or preventing a solid tumor in a patient, comprising administering to the patient an effective amount of a cell hybrid, wherein the patient-derived white blood cell is derived from the patient being treated and the fusion partner expresses a heterologous tumor antigen. The antigen expressed can be selected from the class of cancer-specific antigens, including, but not limited to those specifically named herein.

A method of treating or preventing HIV infection or AIDS in a patient, comprising administering to the patient a cell hybrid, wherein the patient-derived white blood cell is derived from the patient being treated and the fusion partner expresses a heterologous HIV-specific antigen. The antigen expressed can be selected from the class of HIV-specific antigens, including, but not limited to those specifically named herein.

The present invention provides preventive and therapeutic vaccines for cancer or HIV infection, including AIDS, based on irradiated semi-allogeneic cell hybrids, generated by the fusion of patient-derived tumor or white blood cells, with the allo cell provided herein (26). Semi-allogeneic cell hybrids can be inactivated by irradiation and injected into the same patient to induce a specific anti-tumor or anti-HIV response, respectively. The present hybrids eliminate the need to establish patient-derived tumor cell cultures, which notoriously constitute a major technical hurdle. Furthermore, FO-1-derived HLA class I antigens may enhance the anti-tumor or anti-HIV response by virtue of the allogeneic presentation of tumor or HIV antigens; and cell hybrid vaccines exposed to a single lethal dose of ionizing radiation can express HLA class I surface antigens for several days before dying.

The cancer or HIV infection or AIDS prevention or treatment method, wherein the cell hybrid is administered in conjunction with a cytokine is also provided. The cytokine can be interleukin-12 (IL-12), granulocyte-macrophage colony-stimulating factor (GM-CSF), interleukin-2 (IL-2), or a combination of these and other adjuvants.

Administration of the semi-allogeneic hybrid.

Cell hybrids derived from the fusion of FO 1-12 cells with patient-derived melanoma cells or any other cell are selected by virtue of their HAT-resistant and neomycin-resistant phenotype as described below. At the time of vaccination, the hybrids are thawed and used to prepare the irradiated vaccine for injection as described below.

The vaccine consists of $5x\ 10^6$ (or more) irradiated tumor cell hybrids. Irradiated cells are resuspended in 0.1 ml physiological saline and injected intradermally (i.d.) into the surface of the shoulder or other cutaneous area as deemed appropriate by the physician.

Multiple vaccinations may be required to induce immunity. Follow-up vaccinations can be made until complete remission or stabilization is achieved.

Activation of CTL against patient-derived, disease-specific cells.

A method of enhancing the proliferation and activation of a patient's cytotoxic T lymphocytes (CTL) specific for tumor-associated, HIV/AIDS-associated or autoimmune disease-associated antigen targets is provided. The method comprises contacting a population of peripheral blood mononuclear cells (PMBC), such as peripheral lymphocytes, from the patient with a cell hybrid of the present invention for an amount of time sufficient to increase the numbers and cytotoxic activity of CTL in the population. The amount of time can vary, but is expected to be in the range of from one to two weeks. An example of this method is provided in the Examples herein.

As used in the present context, the term "contact" includes close proximity as well as actual mechanical contact.

Because of the ability of the present hybrids to activate CTL, the hybrids can be used in a method of treating a solid tumor in a patient. The method can comprise the steps of: a) obtaining a population of PBMC from the patient; b) contacting the population of PBMC with a cell hybrid of the invention for an amount of time sufficient to enhance the proliferation and activation of the patient's cytotoxic T lymphocytes; and c) returning the population of PBMC to the patient, whereby the tumor is treated.

The step of obtaining a population of lymphocytes from a patient is accomplished by any of the well known methods of obtaining peripheral blood-derived lymphocytes. A specific example of one such method is described in the Examples. The length of contacting time is essentially as described below. The step of returning the activated lymphocyte population to the patient can be by known methods.

Having provided a method of enhancing the proliferation and activation of CTL, the invention also provides a composition comprising a population of cytotoxic T lymphocytes produced by this method. This composition is a valuable reagent in the

screening and identification of tumor-associated, HIV/AIDS-associated, or autoimmune disease-associated antigens or antigenic peptides.

Thus, a method for educating patient-derived lymphocytes to enhance the activation of cytotoxic T lymphocytes specific for patient-derived tumor-associated, HIV/AIDS-associated, or autoimmune disease-associated antigens is provided. The patient-derived lymphocytes are educated by exposing them to irradiated semi-allogeneic cell hybrids derived from the fusion of patient-derived cells with a cell having a selectable dominant marker and having a selectable recessive marker (for example, the cell line FO-1 #12). The patient-derived cell used to form the semi-allogeneic cell hybrids can be a tumor cell or peripheral blood mononuclear cell (PBMC). The patient-derived tumor cell can be a melanoma cell, a prostatic carcinoma cell, a colon carcinoma cell, a lung carcinoma cell, a breast carcinoma cell, a pancreatic carcinoma cell, a prostatic carcinoma cell etc. The patient-derived PBMC can be from patients with cancer, HIV-infection or AIDS, autoimmune disease, etc. An example of this method is described in the Examples.

Screening for tumor-, HIV- or other disease-associated antigens

A method of screening for tumor-associated, HIV/AIDS-associated or autoimmune disease-associated antigens or antigenic peptides is provided. Briefly, CTL can be obtained from the peripheral blood of any patient who responds clinically to any form of the present vaccine; in parallel, B lymphocytes from the same blood sample can be obtained and immortalized by infecting them with Epstein-Barr virus (EBV). Patient-derived CTLs can be activated by exposing them to irradiated, semi-allogeneic cell hybrids (vaccine) and tested for *in vitro* lysis of the patient's own B lymphocytes after they have been mixed with antigenic peptides extracted from the vaccine itself. Biological evidence of antigenic-mediated lysis can be used as a crucial indicator to pursue the identification by routine physical-chemical means (e.g., mass spectrometry) of the sequence of the antigenic peptides eliciting the cytotoxic response.

Thus, a method for using educated cytotoxic T lymphocytes as cellular reagents in the identification of tumor-associated, HIV/AIDS-associated, and autoimmune disease-associated antigens or antigenic peptides is provided.

The present invention is more particularly described in the following examples which are intended as illustrative only since numerous modifications and variations therein will be apparent to those skilled in the art.

EXAMPLE 1

Construction of FO-1 #12 cells

FO-1 human cells are deficient in β_2 microglobulin production; therefore, they do not express HLA class I surface antigens (18). Expression of a transfected human β_2 microglobulin gene in FO-1 cells leads to restored expression of HLA class I antigens (19).

Derivation of hgprt$^-$ FO-1 cells:

FO-1 cells were mutagenized by exposing them to a single dose (3 Gy) of γ-radiation (6 Gy/min dose rate) and subsequently plated in complete medium containing the purine analog 6-thioguanine at a concentration of 5 μg/ml. The incorporation into the DNA allows the selection of cells that are deficient for hypoxanthine-guanine phosphoribosyl transferase (hgprt). Several hgprt-deficient (hgprt$^-$) FO-1 mutants were isolated and characterized for their sensitivity to hypoxanthine, aminopterin, and thymidine (HAT)-containing medium. A particular hgprt$^-$ FO-1 clone with a spontaneous reversion rate to a HAT-resistant (hgprt$^+$) phenotype of $<1x10^{-7}$ was selected. However, the actual reversion rate must be much lower, since after several months of experimentation, not even a single HAT-resistant FO-1 (hgprt$^+$) revertant was found.

Transfection of FO-1 hgprt$^-$ cells:

Exponentially growing FO-1 hgprt$^-$ cells were transfected, using the calcium phosphate precipitation technique (20), with a plasmid containing the neomycin-resistance gene (21). Neomycin-resistant clones were selected in Dulbecco's modified Eagle's medium (DMEM) with added 10% fetal bovine serum (FBS), 50 units/ml penicillin, 50 µg/ml streptomycin, and 40 µg/ml ciprofloxacin (complete medium), containing the neomycin analog geneticin (Gibco) at a concentration of 600 µg/ml. Neomycin-resistant clones became visible 3 weeks after transfection; and individual clones were expanded for further characterization. In another method of raising cells, the medium contained 100 U/ml penicillin, 100 µg/ml streptomycin and no ciprofloxacin.

Subsequently, a cell survival curve for FO-1-neo transfectants exposed to γ-rays was generated. Specifically, single-cell suspensions in complete medium were irradiated with a single dose of γ-rays, ranging from 3 to 20 Gy, using a ^{137}Cs γ-radiator (J.L. Shepherd and Assoc.) delivering 6 Gy/min. Irradiated cells were plated in cell culture dishes with complete medium, and surviving clones were scored two weeks after irradiation by their ability to form colonies, which were counted following fixation and staining with Giemsa (Fig. 1). The result of these cell survival experiments indicated that a single dose of 25 Gy would be sufficient to inactivate ~$1x10^{12}$ cells. Therefore, this radiation dose was selected as a standard for inactivation of cells.

The coexistence in these engineered cells of a dominant marker (e.g., neomycin-resistance) and a recessive mutation (e.g., hgprt$^-$) are necessary and sufficient for the easy selection of semi-allogeneic cell hybrids (e.g., following PEG-mediated fusion of FO-1 transfectants with patient-derived tumor or white blood cells as described below) provided by the present invention.

Although only a single clone (FO-1 #12) was selected for use in the subsequent experimentation, given the teaching of the present specification, it is expected that other

cells having the key characteristics of the exemplary FO-1 #12 cells are within the scope of routine repetition of the above described steps. For example, FO-1 #5, which has selectable dominant and recessive markers and is β_2 microglobulin deficient has also been made, and exhibits comparable biological properties.

Generation of tumor cell hybrids

Polyethylene glycol(PEG)-mediated cell fusion (22) between neomycin-resistant, hgprt$^-$ (HAT-sensitive) FO-1 #12 cells, and patient-derived cells, was conducted according to the procedure by Prado et al (23). When fusing FO-1 #12 cells with patient-derived cells in suspension (e.g., patient-derived white blood cells), the so-called stirring protocol is used (24).

Preliminary experiments of PEG-mediated cell fusion were carried out between FO-1 #12 and 501 human melanoma cells. Neomycin-resistant and HAT-resistant melanoma cell hybrids (FO-1 #12 x 501) were subjected to immunofluorescent staining using anti-HLA-A,B,C + β_2 microglobulin mAb W6-32 and anti-HLA-A2 mAb PA2.1, followed by affinity-isolated fluorescein-labeled goat anti-mouse immunoglobulin (Fig. 2).

The expression by FO-1 #12 x 501 cells of HLA-A2 surface antigen derived specifically from 501 parental cells confirmed that true hybrids had been obtained.

More recently, cell hybrids derived from the PEG-mediated fusion of FO-1 #12 cells with patient-derived tumor cells were generated and characterized. These patient-derived cells were obtained from tumor lesions removed as part of standard surgery and were in excess of the patients' needs. The resulting hybrids expressed HLA class I antigens.

Tumor cell hybrids from over eighty independent experiments of PEG-mediated cell fusion have been obtained, including: two human melanoma cell lines; one human

prostatic carcinoma cell line; over forty patient-derived primary melanoma cell suspensions; two patient-derived primary colon carcinoma cell suspensions; over twenty patient-derived lung carcinoma cell suspension; two white blood cell lines; and over ten HIV-infected patient-derived peripheral white blood cell suspensions.

Survival curves of tumor cell hybrids following exposure to γ-radiation were generated and the results of these studies indicate that tumor cell hybrids are as radiation-sensitive as parental FO-1 #12 cells; therefore, 25 Gy γ-ray (at 6 Gy/min) was adopted as a standard lethal dose to inactivate hybrids for vaccine purposes.

Semi-allogeneic cell hybrids tailored to and specific for each patient can be generated with cell suspensions from any solid tumor or from white blood cells; they can be propagated and irradiated before injecting them into each patient for the purpose of therapeutic as well as preventive vaccination. Moreover, irradiated tumor cell hybrid vaccines can be formulated with appropriate cytokines (IL-12, GM-CSF, IL-2, etc.) for enhanced efficacy.

Derivation of white blood cells (peripheral mononuclear lymphocytes) from peripheral blood:

Peripheral mononuclear lymphocytes (PML) are obtained from 20 ml of heparinized human blood. After diluting blood with Hank's balanced salt solution (HBSS) at a 1:1 ratio, the suspension is layered over the separation medium (Lymphocyte Separation Medium- LMS- Organon Teknika) and spun down at 400xg at room temperature for 15-30 min. Centrifugation sediments erythrocytes and polynuclear leukocytes and bands mononuclear cells which can be aspirated, transferred to a centrifuge tube and diluted with an equal volume of HBSS. The mononuclear lymphocyte suspension is spun down for 10 min at room temperature at a speed sufficient to sediment the cells without damage (i.e., 160-260xg). Cells are washed again in HBSS, resuspended in appropriate diluent and counted before using them for fusion.

Derivation of tumor cell suspensions from surgically excised lesions:

The present protocol is a modification of the tumor disaggregation protocol by Yannelli et al (25). Tumors are retrieved immediately after excision from each patient, put in Hank's balanced salt solution (HBSS) on ice, and transported to the laboratory. Tumor specimens are then transferred under sterile conditions to a 100 mm culture dish containing HBSS. After separating fat and necrotic tissue away from tumor tissue (1-2 grams), the latter is minced into pieces as small as possible using scalpel blades.

Minced tumor tissue fragments are transferred to flasks containing 25-50 ml of an enzymatic solution made of RPMI medium without serum, containing type I collagenase (1.0 mg/ml, Worthington) and DNase I (0.1 mg/ml, Sigma). The flask containing tumor cells is incubated at room temperature for 16-18 hours on a magnetic stir plate. The digested tumor cell suspension is then filtered through a sterile Nitex 40 nylon filter (mesh size 95 μm) to exclude undigested tumor fragments. The cell suspension is transferred to 50 ml conical centrifuge tubes and spun at 250xg for 10 min at 4°C in a refrigerated centrifuge, washed once with HBSS, resuspended in an appropriate volume of HBSS and layered over Lymphocyte Separation Medium (LMS, Organon Teknika) and spun down at 400xg at room temperature for 15-30 min. Centrifugation sediments erythrocytes and polynuclear leukocytes and bands mononuclear blood cells and tumor cells which can be aspirated, transferred to a centrifuge tube and diluted with an equal volume of HBSS. The cell suspension is spun down for 10 min at room temperature at a speed sufficient to sediment the cells without damage (i.e., 160-260xg). Cells are washed again in HBSS, resuspended in appropriate diluent and counted and checked for viability by trypan blue exclusion test. Separate aliquots of the single cell suspension are used for a) fusion with FO-1 #12 cells to derive tumor cell hybrids, b) growth of tumor-infiltrating lymphocytes, and c) freezing for later use as autologous targets in cytotoxicity assays (see below). During processing, all solutions include gentamicin (50 µg/ml).

Formation, propagation, and irradiation of tumor cell hybrids:

The procedure outlined below is a variation of the one reported by Prado et al (23) for the PEG-mediated fusion of somatic cells in monolayers. Thus, a preferred choice for fusing agent is high quality PEG-1450 (purchased from ATCC) which has been pretested for cytotoxicity.

Single-cell suspensions of patient-derived tumor cells ($1x10^7$ cells/100 mm dish) are plated on tissue culture dishes in DMEM supplemented with 10% FBS, streptomycin (100 µg/ml) and gentamicin (10 µg/ml). The following day, $4x10^6$ FO-1 #12 cells are added to each dish of patient-derived cells for co-cultivation. After 4-5 hours of co-cultivation, cells are rinsed twice with serum-free DMEM prewarmed at 37° (D37°), and exposed for 5 min to 50 µM sodium dodecylsulfate (SDS) in D37°. SDS-containing medium is suctioned off and the monolayer is treated with 3 ml/dish of 50% PEG in D37° for fusion. The PEG solution is suctioned off and the monolayer rinsed three times with D37° before adding complete medium containing 15 µg/ml hypoxanthine, 0.2 µg/ml aminopterin, 5 µg/ml thymidine (HAT). The day following PEG-mediated cell fusion, selection for tumor cells hybrids is started in complete medium containing HAT and 600 µg/ml of the neomycin analog geneticin (G418). Cell hybrids derived from the fusion of FO-1 #12 cells with patient-derived cells are selected by virtue of their HAT-resistant and neomycin-resistant phenotype and are propagated in selective medium for several weeks.

When fusing FO-1 #12 cells with cells in suspension [e.g., patient-derived peripheral blood mononuclear cells (PBMC)], a modification of the stirring protocol is used (24). PBMC and FO-1 #12 cells are washed by centrifugation in D37° and then mixed at an approximate 5:1 ratio (25 million PML:5 million FO-1 #12 cells). The resulting cell mixture is then spun at 300xg for 5 min in D37° containing 50 µM SDS. The mixed cell pellet is resuspended in 1 ml 50% PEG added slowly over 1 minute, and then stirred for an additional minute. Next, 10 mls D37° is slowly added over 2 minutes while stirring. The cell suspension is then centrifuged at 300xg for 5 min. The cell pellet is resuspended in complete medium containing 15 µg/ml hypoxanthine, 0.2

μg/ml aminopterin, 5 μg/ml thymidine (HAT). The day following PEG-mediated cell fusion, selection for cell hybrids is started in complete medium containing HAT and 600 μg/ml of the neomycin analog geneticin (G418). Cell hybrids derived from the fusion of FO-1 #12 cells with patient-derived PML are selected by virtue of their HAT-resistant and neomycin-resistant phenotype and are propagated in selective medium for several weeks.

Cell hybrids derived from the fusion of FO 1-12 cells with patient-derived melanoma cells are selected by virtue of their HAT-resistant and neomycin-resistant phenotype and propagated in selective medium for several weeks. The HAT-resistant and neomycin-resistant cell population is then subjected to immunofluorescent staining using anti-HLA class I antigen mAb W6-32, followed by affinity-isolated fluorescein-labeled goat anti-mouse immunoglobulin. Mab W6-32 (corresponding hybridoma obtained through ATCC) is available as sterile ascites obtained from virus-free, immunodeficient (nude) mice and is used as a 1:1000 dilution in staining solution (full reactivity of W6-32 sterile ascites at 1:4000 dilution was documented). The surface expression by HAT-resistant and neomycin-resistant cells of HLA class I antigens confirms the presence of true hybrids. As an additional confirmation, tissue typing of patient-derived white blood cells and tumor cell hybrids is performed.

Determination of sterility and endotoxin activity:

Sterility, mycoplasma and endotoxin testing are initiated on the fin cell hybrid preparation for injection and on the autologous tumor cells and peripheral blood leukocytes used for skin tests. A gram stain is performed on the hybrid cells prior to injection. Mycoplasma testing can be performed utilizing the PCR-based detection kit manufactured by Stratagene (catalog #302007), which allows the identification of any of five strains of mycoplasma commonly associated with cell culture infections. Endotoxin testing can be performed using the Limulus Amebocyte Lysate-based kit (Pyrogent Plus Gel-Clot LAL) manufactured by Bio-Whittaker (Walkersville, MD).

Preparation of irradiated hybrids for vaccination:

Approximately $5x10^6$ cell hybrids (sufficient for 1 injection of vaccine at the minimum dose) are washed three times in HBSS, resuspended in 4 ml of HBSS, tested for viability by trypan blue exclusion (at least 70% viability is preferred), and exposed to a single dose of 25 Gy γ-rays, sufficient to kill all cell hybrids. Irradiated cell hybrids are spun down at ≈ 250xg for 5 min and resuspended in 0.1 ml physiological saline before injection (I.D.). To insure uncompromised vaccine efficacy, the time-lapse between irradiation and vaccine administration should not exceed about two hours. Neomycin-resistant, HLA class I antigen-expressing hybrids are expanded and frozen down in aliquots of $6x10^6$ or more cells.

Samples of cell hybrids from each patient can be identified by some accepted identifier (e.g., the patient's initials followed by their hospital registration number and the letters FO-1).

Derivation of tumor-infiltrating lymphocytes (TIL) and peripheral blood lymphocytes (PBL):

TIL cultures are established as described by Yannelli et al. (25). Initial single-cell suspensions, containing tumor cells, lymphocytes, macrophages, and stromal cells ($5x10^5$ cells/ml), are seeded in 24-well culture plates (2 ml/well) in RPMI (Gibco-BRL) supplemented with 10% human AB serum, streptomycin (100 μg/ml), gentamicin (10 μg/ml), 2 mM L-glutamine, and interleukin-2 (IL-2, Cetus-Chiron, 6000 IU/ml). After 5-7 days, when the cell densities exceed $1.5x10^6$ cells/ml, cultures are expanded and transferred to tissue culture flasks at a concentration of $5x10^5$ cells/ml in fresh medium. After 2-3 weeks, TIL cultures are tested for the surface expression of T cell markers (MHC class II, CD3, CD4, CD8) by flow cytometry using commercially available reagents (Coulter). TIL are cryopreserved in aliquots of $2x10^7$ cells/vial and stored in liquid nitrogen until use.

To obtain peripheral blood-derived lymphocytes (PBL), mononuclear cells are obtained from heparinized blood as described above and grown in AIM-V (Gibco-

BRL) supplemented with 10% human AB serum, streptomycin (100 μg/ml), gentamicin (10 μg/ml), 2 mM L-glutamine, and interleukin-2 (IL-2, Cetus-Chiron, 6000 IU/ml) for 1-2 weeks. The cells can then be tested for the expression of T cell markers (MHC class II, CD3, CD4, CD8) by flow cytometry using commercially available reagents (Coulter) before using the cells in experiments of "education" with semi-allogeneic cell hybrids.

Education of patient-derived TIL or PBL and cytotoxicity studies:

For experiments of "education" of patient-derived TIL or PBL, semi-allogeneic cell hybrids derived from the same patient ($1x10^6$ cells in 3 ml of complete medium) are irradiated (25 Gy) and plated onto a 100 mm culture dish. After the cells are attached, medium is suctioned off and replaced with $5x10^6$ TIL or PBL in 10 ml of AIM-V medium without serum, with or without 120 IU/ml IL-2. As controls, identical cultures of TIL or PBL either exposed to irradiated parental FO-1 β_2 microglobulin$^+$ cells, or not exposed to any irradiated cells are established. After three to seven days, all three sets of TIL or PBL cultures are tested for cytolytic activity against ^{51}Cr-labeled autologous target cells in ^{51}Cr release assays.

Depending on the experiment, target cells can be patient-derived tumor cell suspensions, patient-derived peripheral blood mononuclear cells (for example, in patients with HIV/AIDS or autoimmune disease), Epstein-Barr virus (EBV)-transformed B lymphocytes loaded with appropriate antigenic peptides (26), or other HLA-matched antigen-presenting cells, such as T2 cells.

Target cells are radiolabeled with ^{51}Cr in complete RPMI medium over a one to two hour period or overnight, depending on cell type. The targets are then incubated with the educated lymphocytes or controls in complete RPMI medium for 4-16 hours at effector to target ratios of 5:1 and 40:1. ^{51}Cr release into the supernatants is measured by a gamma counter. The percent lysis (% lysis) from the cytolysis assays is calculated by the following formula:

$$\% \text{ Lysis} = \frac{Ex - S}{M - S} \times 100$$

where Ex = experimental release of ^{51}Cr (cpm/min), S = spontaneous release of chromium-51 (cpm/min) by target cells, and M is maximum release of ^{51}Cr (cpm/min) by target cells when lysed by 0.1 N hydrochloric acid. As negative controls for the target cells, ^{51}Cr-labeled Daudi cells were used as targets for lymphokine-activated killer (LAK) cell activity and ^{51}Cr-labeled K562 cells as targets for natural killer (NK) cell activity, in order to ascertain that any change in cytotoxicity after exposure of lymphocytes to irradiated hybrids was T cell-mediated rather than being the result of increased LAK or NK cell activity (LAK and NK cell activities are not HLA-restricted). The results of cytotoxicity experiments are shown in Tables 1 and 2. Values representing percent lysis are corrected for the percent lysis by each effector of ^{51}Cr-labeled Daudi cells used as a non-specific target.

Table 1 shows percent of TIL-mediated lysis of autologous melanoma cells (target) from patient JP1. Values shown were corrected for the % lysis by each effector of Daudi cells used as a target for lymphokine-activated killer (LAK) cells. [a]ND: not determined (JP1-TIL were growing poorly in the absence of stimulation with irradiated hybrids).

Table 1

	EFFECTOR:TARGET RATIO	
EFFECTORS	**5:1**	**40:1**
JP1-TIL (control + IL-2)	0.0	ND[a]
JP1-TIL (JP1 x FO1-educated)	11.0	67.0
JP1-TIL (JP1 x FO1-ed. + IL-2)	36.5	75.0
	% LYSIS OF AUTOLOGOUS TUMOR CELLS	

Table 2 shows the percent of TIL-mediated lysis of autologous melanoma cells (target) from patient GT1. In this experiment, a control was performed with GT1-TIL exposed to irradiated F0-1 parental cells transfected with the β_2 microglobulin gene. These FO1-β cells express allogeneic MHC class I molecules on the cell surface. This experiment demonstrates that allogeneic stimulation per se does not enhance specific cytolytic activity by the TIL as does the semi-allogeneic stimulation by the GT1xFO1 hybrids. Values shown were corrected for the % lysis by each effector of Daudi cells used as a target for LAK cells.

Table 2

	EFFECTOR:TARGET RATIO	
EFFECTORS	**5:1**	**40:1**
GT1-TIL (control + IL-2)	5.0	19.2
GT1-TIL (FO1β-ed. + IL-2)	12.9	21.2
GT1-TIL (GT1 x FO1-ed. + IL-2)	16.0	42.7
	% LYSIS OF AUTOLOGOUS TUMOR CELLS	

EXAMPLE 2

Patients

HIV^+ (HIV-positive) patients were enrolled in a joint study of a Department of Radiation Oncology, and the Division of Infectious Disease, Medical University of South Carolina, Charleston, SC, USA. Whole blood was collected at three monthly intervals and shipped overnight to the NCI for *in vitro* experiments. The study was reviewed and approved by Institutional Review Boards from both Institutions. Most HIV^+ patients tested had low detectable viral loads (<400 copies/ml) and $CD4^+$ counts above 250 cells/ml. The information on viral loads, $CD4^+$ and $CD8^+$ counts, and

antiretroviral therapy from the six patients who were tested for CTL are presented in Table 3.

Table 3. Clinical data of the six HIV^+ patients who were analyzed for *env*-specific CTL.

Patient number	Plasma HIV RNA (copies/mL)	CD4 count (mm^3)	Antiretroviral therapy
1	<400	413	3TC, Indinavir
2	<400	376	No therapy
3	<400	746	AZT, 3TC, Nelfinavir
4	<400	420	AZT, 3TC
5	1486	508	No therapy
6	<400	276	AZT, 3TC, Nelfinavir

Antiretroviral therapy included: AZT, 3TC (Lamivudine), IND (Indinavir), and ddL (Didanosine) and Nelfinavir.

Preparation of Peripheral Blood Mononuclear cells

Whole blood was collected into heparinized vacuum tubes (Becton Dickinson Vacutainer Systems, Franklin Lakes, NJ) in Charleston, coded and shipped overnight at ambient temperature to the Bethesda laboratory. The PBMC were separated by centrifugation at 2000 rpm for 30 minutes on Ficoll-Hypaque gradients and were washed twice in PBS (Gibco BRL, Grand Island, NY) and resuspended in DMEM medium supplemented with 2mM L-glutamine, 100 U/ml penicillin, 100 μg/ml streptomycin and Hepes buffer (Gibco) (4).

Fusion and irradiation of hybrids

PEG-mediated fusion was performed according to a modification of the procedure described by Galfre (24). PEG-1450 was used as a fusion agent (Sigma, St. Louis, MO). Briefly, PBMC from donors (2.5 X 10^7 cells/ml) and the allogeneic melanoma line FO1-12 (5 X 10^6 cells/ml) were washed and centrifuged at 1000 rpm in DMEM without serum. The cell pellet was resuspended in medium without serum containing 50 μM SDS (Sigma), and PBMC were mixed with FO1-12 line at 5:1 ratio and spun at 1200 rpm. The mixed cell pellet was slowly resuspended in 1 ml 55% PEG for 1 minute. Medium with serum was added slowly over 2 minutes while stirring. The cell suspension was centrifuged at 1000 rpm and cells resuspended in DMEM medium with 10% FBS. HAT was added to medium 24 hr after fusion and geneticin (600 μg/ml) was added 48 hr after fusion. Media was changed 3 times per week and cells were passaged as needed. The HAT-resistant and neomycin-resistant hybrids were tested for surface expression of HLA class I antigens by immunofluorescence using monoclonal antibodies to Human class I antigens (Pharmingen, San Diego, CA). Tissue typing of donor PBMC and hybrids was performed by molecular typing using PCR technique in the NIH HLA Laboratory (Bethesda, MD).

Survival curves of these semi-allogeneic cell hybrids were generated following exposure to γ radiation, and the results verified the radiation-sensitivity of semi-allogeneic hybrids to be 25 Gy (corresponding to a surviving fraction of ~1x10^{-12}) as noted in Example 1.

Cytokine production and detection

The production of cytokines by semi-allogeneic hybrids or FO1-12 line was assessed by culturing 1.5 X 10^6 cells/ml in DMEM medium with 10% FBS in 24-well plates in a humidified, 37°C, 5% CO_2 incubator. Cells were unstimulated and were either unirradiated or irradiated (25 Gy). Culture supernatants were harvested after 24 and 48 hr and stored at -80°C. Total IL-12 p40, IL-2, IL-4, IL-6 production were determined by ELISA (Genzyme, Cambridge, MA). IL-10, IL-5, IFN-γ, and TNF-α

production were assessed using Pharmingen capture and detection antibodies (Pharmingen). The IL-12 p70 heterodimer production and GM-CSF release were assessed by ELISA (R&D, Minneapolis, MN).

Surface molecule detection by immunostaining

Hybrids and FO1-12 cells were washed three times with staining buffer (PBS plus 0.1% BSA). Approximately 10^6 cells were incubated with human IgG (20 μg/ml) for 10 minutes at 4^0C to block Fc receptors, and than stained with FITC or PE conjugated mAb W6-32 (anti-HLA-A,B,C+β_2-microglobulin), anti-CD80 (B7.1), anti-CD86 (B7.2), anti-HLA DR, anti-HLA DQ (Becton Dickinson, Mountain View, CA) or with isotype-matched Ab for 30 minutes at 4^0C. Cells were washed twice and resuspended in staining buffer and surface molecule expression was determined by FACS analysis using a FACScan (Becton Dickinson) and CellQuest software.

Synthetic peptides

The HIV-1 envelope peptides used in this study were synthesized as previously described (36-38), and are identical to those previously reported in HIV^+ patients studies by our laboratories (39, 40). The peptides based on sequence of gp160 HIV-1 IIIB are: *env* T1 (aa residues 428-443:gp120); *env* T2 (aa residues 112-124: gp120): *env* Th4.1 (aa residues 834-848: gp160) and *env* P18 (aa residues 315-329: gp160). An *env* peptide based on sequence of gp160 HIV-1 MN was also used (P18 MN, homologous to P18 IIIB; gp160). Peptides were dissolved in DMEM medium and stored at -80 ^{0}C.

Stimulation of PBMC

PBMC were separated from heparinized blood samples by density gradient centrifugation and washed three times in RPMI 1640 medium. PBMC (3 X 10^6 cells/ml) were cultured in triplicate in flat bottom 96-well microtiter plates in a final volume of 200 μl of complete medium supplemented with 10% heat activated AB^+ serum (Hyclone). Donor PBMC were stimulated with either irradiated (25 Gy) allogeneic cells or semi-allogeneic hybrid cells (at responder: stimulator ratio 3:1) or

env peptide pool (5μM of each peptide) for 6 days as previously described (18, 33). Cultures were pulsed with 1 μCi ^{3}H-thymidine and harvested using a Basic 96 Harvester (Skatron Instruments, Sterling,VA), and the radioactivity was counted with a β-counter (Wallace, Gaithersburg, MD).

Cytotoxic T cell generation and assay

Responder cells. 3 X 10^6 PBMC were incubated for 6 days with HIV *env* peptide pool (5 μM) at 37^{0}C in a humidified 5% CO_2 incubator in RPMI 1640 medium supplemented with 5% human AB$^+$ serum. The cells were washed and resuspended in RPMI 1640 medium with 5% human AB$^+$ serum and used in a CTL assay.

Semiallogeneic hybrids were washed and irradiated (25 Gy) and used as stimulators for 6 days with responder cells at a ratio 1:3.

Stimulation with *env* peptides. The pool of five *env* peptides were added to the PBMC culture at 5 μM concentration for each peptide during the six day stimulation of CTL. In one experiment (see Fig. 7) :1) PBMC were pulsed with *env* peptides for 1 hr at 37^{0}C, washed and then mixed with irradiated semi-allogeneic hybrids; or 2) irradiated semi-allogeneic hybrids were pulsed with *env* peptides for 1 hr at 37^{0}C, washed and then mixed with PBMC. Both 1) and 2) above were cultured for six days to generate *env*-specific CTL.

Target cells. Target cells were EBV-transformed B lymphoblastoid cell lines. Autologous EBV lines were generated incubating PBMC with the supernatant of B95.8 cells, a cell line that chronically produces Epstein Barr virus, and an anti-CD3 monoclonal antibody (Pharmingen). Target cells were pulsed overnight with either *env* peptide pool (5 μM each) or no peptides. After three washes the targets were labeled with Chromium-51 (150 μCi $Na_2{}^{51}CrO_4$; Amersham, Piscataway, NJ) for 2 hr at 37°C. The targets were resuspended at a 5 X 10^3 cells/well in RPMI 1640 containing 5% human AB$^+$ serum and incubated in 96-well round bottom microtiter plates in triplicates at effector: target ratio 30:1, 10:1, 3:1, and 1:1. Spontaneous release

was determined in targets cultured in medium alone. Maximal release was determined from targets cultured with 5% Triton X-100. After 6 hr of incubation, supernatants were harvested and counted in a γcounter. Percent specific lysis was determined as 100% X (experimental cpm- spontaneous cpm)/(maximum cpm-spontaneous cpm). "Effector" cells are cytotoxic T lymphocytes (CTL) that lyse (kill) target cells. Effector cells are generated from cytotoxic precursor cells by stimulator cells that can be peptide-loaded autologous cells or in this invention also peptide-loaded hybrid cells. "Peptide-loaded" is the same as "peptide-pulsed."

Generation of donor-derived, semi-allogeneic hybrids

FO1-12 cells, which express class II HLA antigens, were used as an allogeneic donor cell line for preparing semi-allogeneic cell hybrids with uninfected donor (HIV^-) or HIV^+ patient-derived cells. The co-existence in FO1-12 cells of a dominant-selectable marker (neomycin-resistance) and a recessive-selectable marker (HAT-sensitivity; $hgprt^-$), allows for the easy selection of cell hybrids (following PEG-mediated fusion of FO1-12 cells with PBMC) in HAT medium containing the neomycin analog G418. FO1-12 cells are also deficient in β_2-microglobulin production, because of the deletion in the corresponding gene, and do not express HLA class I surface antigens (18). Thus, expression of class I on the hybrid cells provides additional evidence of fusion and verifies co-expression of patients' self-MHC class I. Hybrid cells generated by fusion with FO1-12 express HLA class I surface antigens derived from both FO1-12 and the patient, due to the expression of the patient-derived β_2-microglobulin gene. The neomycin-resistant/HAT-resistant cell hybrids were tested for HLA class I expression by immunostaining and flow cytometry. The percentage of HLA class I-expressing cell hybrids was greater than 90%, and the hybrids were stable in culture over time in terms of the percentage class I-expressing cells. These three markers greatly simplify the screening of semi-allogeneic cell hybrids, and were used to confirm hybrid formation.

To further demonstrate that hybrids had been formed that expressed the class I antigens of both patient and FO1-12, the hybrid cell lines were subjected to molecular HLA haplotype analysis by the NIH HLA Laboratory, including molecular typing of FO1-12 cells expressing the β_2-microglobulin gene (FO1-β_2) (19) (Table 4). PBMC from the patients were also analyzed for HLA class I expression. All of the hybrids tested expressed HLA class I and II antigens derived from both fusion partners.

Table 4. HLA class I and II antigen expression by FO1-12 line; FO1-β2 line expressing a transfected β_2-microglobulin gene (19); PBMC and hybrid cell lines from six HIV$^+$ patients analyzed for CTL activity.

Cell Source	Class I	DRB1	DQB1	DRB
FO1-12	None	0301	02	3*0101
FO1-β2	A25, A32, B8	0301	02	3*0101
PBMC-1	A2301, A74, B7, B1503	0804, 1304	0301, 05	3*02
Hybrid-1	A25, A74, B7	0301, 1304	02, 05	3*0101, 3*02
PBMC-2	A1, A2, B8, B44	0301, 0401	0301, 05	3*0101, 4*01
Hybrid-2	A1, A25, B8, B44	0301, 0401	02, 05	3*0101, 4*01
PBMC-3	A30, B8, B57	1304, 01	0301, 0501	3*02
Hybrid-3	A30, A32, B8	0301, 01	02, 0501	3*0101, 3*02
PBMC-4	A6601, A68, B35, B53	15	06	5*01

Cell Source	Class I	DRB1	DQB1	DRB
Hybrid-4	A25, A68, B8	0301, 15	02, 06	3*0101, 5*01
PBMC-5	A32, A33, B5801, B8101	12, 1304	0301, 0501	3*0101, 3*02
Hybrid-5	A25, B8, B8101	0301, 12	0501, 02	3*0101, 3*02
PBMC-6	A3, A74, B7, B1401	1001, 1501	0501, 06	5*01
Hybrid-6	A32, A74, B7	1001, 0301	0501, 02	3*0101, 5*01

Antiretroviral therapy included: AZT, 3TC (Lamivudine), IND (Indinavir), and ddL (Didanosine) and Nelfinavir.

All six hybrid cell lines expressed the class I type of the FO1-12 cell line and, therefore, had the potential of being recognized as allogeneic by T cells from all the patients. With the exception of patient #2, HLA-DR antigens expressed by the hybrids could be recognized as allogeneic HLA class II.

Studies of semi-allogeneic hybrids

Six hybrid lines from HIV^+ donors' PBMC were generated (Table 2), as well as five hybrid lines from HIV-negative (HIV^-) donors' PBMC. There were no detectable differences in the expression of HLA antigens or other surface molecules between HIV^+ and HIV^- donors. The FO1-12 cell line and hybrids were tested for HLA class II expression, both by molecular typing and by immunostaining and flow cytometry. The FO1-12 line and its hybrids expressed the genes for both HLA DR and HLADQ molecules. As with HLA class I expression, the hybrids expressed HLA class II antigens from both parental cells (Table 4). We also performed immunostaining for HLA DR and DQ antigens and found that HLADR, but not HLADQ, molecules were expressed on cell surface of the hybrids (Table 5). All six hybrid cell lines expressed class I of the FO1-12 cell line and had the potential of being

recognized for allogeneic class I by T cells from all of the patients. With the exception of patient #2, some HLA-DR antigens expressed by the hybrids could be recognized as allogeneic HLA class II. The semi-allogeneic hybrids also expressed the costimulatory molecule CD86 (B7.2) (Table 5).

Table 5. Expression of HLA-I, HLA-II (DR) and costimulatory molecules (CD86) was measured for six semi-allogeneic hybrids and allogeneic lines FO1-12 and FO1-12β by flow cytometry analysis. FACS data are presented as mean log fluorescence intensity gating against samples labeled with irrelevant isotype-matched control antibodies (showed in brackets).

Cell cultures	HLA-I	HLA-DR	CD86
FO1-12	5.8 (6.1)	59.9 (4.8)	279.9 (38.4)
FO1-12β	355.1 (4.4)	43.8 (4.9)	189.7 (32.9)
Hybrid-1	385.7 (10.2)	23.2 (2.8)	231.6 (51.8)
Hybird-2	518.6 (13.8)	36.3 (8.4)	173.3 (34.3)
Hybird-3	233.3 (11.3)	41.9 (11.3)	300.5 (74.6)
Hybird-4	283.9 (5.4)	54.1 (7.8)	471.5 (74.2)
Hybird-5	248.9 (14.2)	44.2 (10.2)	291.6 (71.5)
Hybird-6	332.9 (7.8)	35.4 (5.4)	334.5 (106.1)

To study the effect of semi-allogeneic stimulation on T cell function, we cultured PBMC from three patients with irradiated FO1-12 and semi-allogeneic hybrids for five days. An increase in proliferation (3-to-10-fold) was observed after stimulation (Fig. 7). All three donors tested responded weakly to HIV-1 *env* peptide pool stimulus, but responded strongly to FO1-12, and generated intermediate proliferative responses to their respective hybrid cell lines.

Because cytokine gene-transduced tumor cells can exhibit elevated cytokine production after irradiation (41, 42), we compared cytokine production before

irradiation and 24 and 48 hr post irradiation. An increase in interleukin-2 (IL-2) production (4- to -8-fold) was observed in all hybrids tested 24 hr (Fig. 5A) and 48 hr post irradiation. However, if the hybrids were treated with mitomycin C (Sigma, 200 μg/ml for 2 hr at 37°C), no IL-2 release was detected (Fig. 5A). We also tested for other cytokines (IL-4, IL-5, IL-6, IL-10, IL-12, IFN-γ, TNF-α , GM-CSF), and marginal increases of only IL-5 (2-fold) and IL-6 (< 2-fold) were induced by irradiation.

Semi-allogeneic hybrid generation of HIV-specific CTL

Cytotoxic T cell responses were evaluated in six HIV^+ donors. Each patient's PBMC were stimulated with the *env* peptide pool alone or the pool plus semi-allogeneic hybrid derived from that patient to induce expansion and differentiation of HIV-specific CTL. The PBMC were stimulated with *env* pool alone or with semi-allogeneic hybrids alone to obtain control values. PBMC cultures were assayed six days later for cytotoxic activity against EBV- transformed autologous B lymphoblastoid cell lines pulsed with the *env* peptide pool or with media (specificity control) in a 6-hr ^{51}Cr-release assay. PBMC from three of the six HIV^+ donors tested generated cytolytic activity against peptide-pulsed targets (Fig. 6, patients #3, #4, and #6, upper panels) when co-stimulated with *env* peptides plus the respective haplo-identical hybrid cell line. Lysis of target cells required the presence of *env* peptides, as (with one exception) there was no lysis of autologous EBV targets cells that had not been pulsed with peptides (Fig. 6, lower panels). One donor of six (Fig. 6, patient #3) exhibited some HIV-stimulated lysis of unpulsed EBV targets, but it was less than that seen using autologous *env*-pulsed targets. The two other patients exhibited CTL activity that was completely *env*-specific (Fig. 6; patients #4 and # 6). Higher HIV-specific lysis was obtained when PBMC were stimulated with semi-allogeneic hybrids plus *env* peptides compared to stimulation with either peptides or semi-allogeneic hybrids alone. Thus, the data of these three HIV^+ patients (Fig. 6; patients #3, #4, and #6) demonstrate that stimulation of donor PBMC with semi-allogeneic hybrids increased HIV-specific lysis. Patient #1, is a representative

example of the three patients whose PBMC did not generate HIV-specific CTL, even when stimulated with *env* peptides plus semi-allogeneic hybrids.

Irradiation of the hybrids can result in significant increase in IL-2 production by the hybrids (Fig. 5A) and IL-2 has been shown to enhance virus-specific CTL responses (30). Therefore, we compared the amount of *env*-specific CTL activity generated by stimulation with *env* plus irradiated semi-allogeneic hybrids with that generated by *env* plus mitomycin C treated hybrid cells. No difference was observed in CTL activity (Fig. 5B), demonstrating that IL-2 produced by the irradiated hybrid stimulation did not detectably contribute to anti-*env* CTL activity.

Whether the enhancing effect of alloantigen co-stimulation would be more efficient by simultaneous presentation on the same APC of allogeneic MHC with self-MHC plus *env* peptides (on the same APC) or by presentation of alloantigens by the hybrid and *env* by self-APC contained in the patient's PBMC (on separate APC) (Fig. 7) was also tested. To perform this comparative experiment, PBMC were pulsed in one group with *env* peptides for 1 hour, and unbound peptide was washed out before adding the irradiated hybrid cells as co-stimulators. In this group, the alloantigens (hybrid) and *env* plus self-MHC (self-APC in the PBMC) are presented on separate APC (illustrated in Fig. 8A). Alternatively, the irradiated hybrid cells were similarly pulsed with *env* peptides and washed prior to adding PBMC. In this group the alloantigens and *env* plus self-MHC are expressed on the same APC (hybrid) (illustrated in Figure 8 B). The two *env*-specific CTL responses generated were equivalent, and were indistinguishable from the CTL response generated by PBMC plus hybrid cells that were incubated with *env* peptides for the six days (Fig. 7). Control groups for absence of semi-allogeneic hybrids include PBMC stimulated only with *env* for 6 days, and PBMC pulsed with *env* and washed prior to 6 day culture. The *env*-specific T cell activity was 5-to-10-fold lower in PBMC stimulated with *env* only than with *env* plus hybrid cells (Fig. 7). The control group stimulated with hybrid alone was 30-fold below the other groups, confirming that semi-allogeneic stimulation alone did not result in the activation of *env*-specific CTL activity.

The present study demonstrates that hybrid cell lines formed by the fusion of the FO1-12 melanoma line with HIV$^+$ patients' PBMC express class I and II MHC antigens of both fusion partners, and also express at least one of the costimulatory molecules (CD86) necessary for providing the second signal in T cell activation. These hybrid lines can also stimulate T cell proliferation and can present *env* peptides to patients' CTL precursors and provide alloantigen-driven costimulation, resulting in the generation of *env*-specific CTL. The fact that we can increase *env*-specific CTL activity may be important, as it is considered that the Th response to HIV antigens is possibly the first response to be lost after HIV infection (43).

The present study is also the first to demonstrate the costimulatory effect in which human allogeneic and self-HLA determinants are both expressed on the same APC resulting in HIV-specific CTL activity.

The expression of HLA class I provided convenient immunologic evidence of successful fusion. Our findings that the semi-allogeneic hybrids expressed HLA class I and II antigens derived from both cells of origin and also expressed CD86 suggests that the semi-allogeneic hybrids can serve as APC and provide costimulatory signals. The expression of co-stimulatory molecules may be important, because macrophages derived from PBMC of AIDS patients are deficient in CD80/86 expression (45).

In summary, costimulation of T cells from HIV$^+$ patients with haplo-mismatched hybrids can increase *env*-peptide-specific CTL activity relative to the CTL activity generated by *env* peptides without alloantigen costimulation. No additional enhancement was demonstrated *in vitro* by having the allogeneic (helper) and self-restricting (effector) HLA antigens expressed on the same APC, compared to the expression of allogeneic and self MHC antigens on separate APC in the same well. This study demonstrates that hybrid cell lines formed between the patient's PBMC and a genetically-engineered human melanoma cell line can present peptide antigens that stimulate HIV *env*-specific CTL.

EXAMPLE 3

CLINICAL STUDY

OBJECTIVES

The primary objectives of this clinical study are to determine whether treatment with irradiated, semi-allogeneic cell hybrids mixed with HIV-derived peptides is associated: a) with restoration or improvement of delayed-type hypersensitivity (DTH) response to certain recall antigens (mumps and candida) and to allo stimulation (the patient's own semi-allogeneic hybrids); and b) with the ability of PBMC to specifically lyse immortalized B lymphocytes (from the same patient) expressing HIV-derived antigenic peptides.

Secondary objectives of this clinical study are: a) to monitor the patient's profile of CD4 counts and viral load for the duration of the clinical study; b) to identify patients who express HLA-A2 antigen in order to test their peripheral mononuclear cells (PBMC) for the ability to recognize specific, HLA-A2-restricted epitopes derived from influenza A virus and HIV-1; and c) to measure the release of soluble factors with anti-HIV activity by patient-derived PBMC when exposed to semi-allogeneic hybrids.

Generation and characterization of donor-derived, semi-allogeneic hybrids

FO1-12 cells (35), which express HLA class II antigens, were used as an allogeneic donor cell line for preparing semi-allogeneic cell hybrids with patient-derived cells. The co-existence in FO1-12 cells of a dominant selectable marker (neomycin-resistance) and a recessive selectable marker (HAT-sensitivity; hgprt$^-$), allows for the easy selection of cell hybrids (following PEG-mediated fusion of FO1-12 cells with patient-derived PBMC) in HAT medium containing the neomycin analog G418. FO1-12 cells are also deficient in β_2-microglobulin production because of a deletion in the corresponding gene, and they do not express HLA class I antigens on the cell surface (18). Thus, expression of class I on the hybrid cells provides additional evidence of fusion, due to the presence of the patient-derived β_2-microglobulin gene. The neomycin- and HAT-resistant cell hybrids were tested for HLA class I expression

by immunostaining and flow cytometry. The percentage of HLA class I-expressing cell hybrids was greater than 90%, and this percentage was stable in culture over time. Together, these three markers greatly simplify the selection and screening of semi-allogeneic cell hybrids.

FO1-12 cells were certified for human use, and a Master Cell Bank (manufactured for us by Magenta Corporation/Microbiological Associates, Rockville, MD) was utilized for the generation of semi-allogeneic cell hybrids for subsequent clinical studies (see below). Semi-allogeneic hybrids express the class I antigens of both patient and FO1-12 cells, as demonstrated by serological HLA haplotyping (Table 6).

Table 6. HLA class I haplotypes expressed by semi-allogeneic tumor cell hybrids. HLA class I tissue-typing was performed using FO-1 cells expressing a transfected β_2-microglobulin gene (FO1-12 haplotype) (19); patient-derived tumor-infiltrating lymphocytes (patient haplotype); and hybrids resulting from the fusion of FO1-12 cells with patient-derived tumor cells (hybrid haplotype). The patient samples shown represent three melanomas and two lung adenocarcinomas.

FO1-12 Haplotype	Patient Haplotype	Hybrid Haplotype
A25, A32, B8	A11, A29, B35, B44	A25, A29, A32
	A1, A2, B22, B37	A1, A2, A25, A32, B22, B37
	A3, A10, B7, B8	A25, A32, B8
	A11, A36, B55, B35	A25, B22 (55)
	A3, A31, B7, B60, Bw6	A3, A25, B22 (55), B60?

In additional studies (58), six hybrid lines from HIV^+ donors' PBMC (Table 5) were generated, as well as five hybrid lines from HIV-negative (HIV^-) donors' PBMC. The FO1-12 cell line and hybrids were tested for HLA class II expression, both by molecular typing and by immunostaining and flow cytometry. The FO1-12 line and its hybrids expressed the genes for both HLA-DR and DQ molecules. As with HLA class

I expression, the hybrids express HLA class II antigens from both parental cells. Immunostaining for HLA-DR and -DQ antigens showed that only HLA-DR molecules were expressed on the cell surface of the hybrids.

Semi-allogeneic hybrid generation of HIV-specific CTL

Cytotoxic T cell responses were evaluated in six HIV^+ donors (58). In an attempt to induce expansion and differentiation of HIV-specific CTL, each patient's PBMC were stimulated with an HIV-1 *env* peptide pool alone or the pool plus semi-allogeneic hybrids derived from that patient. As controls, PBMC were stimulated with *env* pool alone or with semi-allogeneic hybrids alone. PBMC cultures were assayed six days later for cytotoxic activity against EBV-transformed autologous B lymphoblastoid cell lines pulsed with the *env* peptide pool or with media in a 6-hr ^{51}Cr-release assay. PBMC from three of the six HIV^+ donors tested exhibited cytolytic activity against peptide-pulsed targets [Fig. 9, upper panels, patients #3 (panel A), #4 (panel D), #6 (panel C)] when co-stimulated with *env* peptide plus the respective haplo-matched hybrid cell line. With one exception (patient #3, panel A), lysis of target cells required the presence of *env* peptides, as there was no lysis of autologous EBV target cells that had not been pulsed with peptides (Fig. 9, lower panels). Higher HIV-specific lysis was obtained when PBMC were stimulated with semi-allogeneic hybrids plus *env* peptides compared to stimulation with either peptides or hybrids alone. Thus, the data of these three HIV^+ patients (Fig. 9; patients #3,#4,#6; panels A, D, C, respectively) showed that stimulation of donor PBMC with semi-allogeneic hybrids increased HIV-specific lysis. Patient#1 (Fig. 9, panel B) is an example of the three patients whose PBMC did not generate HIV-specific CTL even when stimulated with *env* peptides plus semi-allogeneic hybrids.

Whether the enhancing effect of alloantigen co-stimulation would be more efficient by simultaneous presentation on the same cell that presented self-MHC plus *env* peptides was tested (Fig. 10) (58). In this comparative experiment, PBMC in one group were pulsed with *env* peptides for one hour, and unbound peptide was washed out before adding the irradiated hybrid cells as co-stimulators. In a second group, the

irradiated hybrid cells were similarly pulsed with *env* peptides and washed prior to adding PBMC. The two *env*-specific CTL responses generated were equivalent, and were indistinguishable from the CTL response generated by PBMC plus hybrid cells that were incubated with *env* peptides for the entire six days. In addition, the cytokines produced by irradiated hybrids do not provide beneficial effect over mitomycin C pre-treated hybrids that do not produce cytokines.

Taken together, these results indicate that hybrid cell lines formed by the fusion of FO1-12 melanoma cells with HIV^+ patients' PBMC can present *env* peptides to patients' CTL precursors and provide alloantigen-driven co-stimulation, resulting in the generation of *env*-specific CTL.

Generation of Semi-allogeneic Hybrids from FO1-12 Cells and Patient-Derived PBMC

Patient-derived PBMC are obtained from approximately 25 ml of heparinized whole blood. Blood is diluted with Hanks' balanced salt solution (HBSS), layered over lymphocyte separation medium (LSM, Organon Teknika), and centrifuged at 400 g for 15-30 min. Centrifugation through this ficoll gradient sediments erythrocytes and polynuclear leukocytes, while an interface band of mononuclear cells can be removed, washed twice with HBSS, resuspended in Dulbecco's modified Eagle medium (DMEM), counted and checked for viability by trypan blue exclusion. Approximately $2.0x10^7$ PBMC are used for fusion with FO1-12 cells (35). About 10% of PBMC are utilized to determine if the patients are HLA-A2+ by immunostaining with anti-HLA-A2 mAb PA2.1. In addition, two aliquots of $2.0x10^6$ cells each are cryopreserved in 90% FBS/10% DMSO for later use as a control for DTH skin tests.

For the generation of semi-allogeneic cell hybrids, a modification of the so-called "stirring" method is used (59, 60). Patient-derived PBMC and FO1-12 cells (obtained from the certified Master Cell Bank) are mixed in serum-free DMEM at approximately a 5:1 ratio (e.g., $2.0x10^7$ PBMC:$4x10^6$ FO1-12), centrifuged at 250g, resuspended and incubated for 5 min in serum-free medium containing 50 μM SDS.

The cell mixture id then centrifuged at 250g and resuspended in 1 ml 50% PEG in serum-free DMEM (added slowly with constant stirring over a 1 minute period). After stirring for 1 additional minute, 10 ml serum-free DMEM are added slowly with constant stirring over a 2 minute period. The cell suspension will then be centrifuged at 250g and plated in a tissue culture flasks with DMEM/10% FBS containing HAT supplement. The day following PEG-mediated cell fusion, selection for cell hybrids is started with complete medium containing HAT and 600 μg/ml geneticin.

Cell hybrids derived from the fusion of HAT-sensitive, neomycin-resistant FO1-12 cells with patient-derived PBMC are easily selected by virtue of their HAT-resistant and neomycin-resistant phenotype and are subsequently expanded in selective medium. Since FO1-12 cells fail to express β_2-microglobulin because of a deletion in the corresponding gene, they do not express HLA class I surface antigens. However, patient-derived β_2-microglobulin expression in the hybrids complements the lack of β_2-microglobulin expression by FO1-12 cells; therefore, the HAT-resistant and neomycin-resistant cell population is tested for the surface expression of HLA class I antigens by immunofluorescent staining using anti-HLA class I mAb W6-32, followed by fluorescein-labeled secondary antibody. The surface expression by HAT-resistant and neomycin-resistant cells of HLA class I antigens confirms that we have obtained true hybrids.

Cell hybrids can easily be propagated to expand their numbers to cryopreserve several aliquots of ~$1.0x10^7$ (or more) cell hybrids in a solution of 90% FBS/10% DMSO. Samples of cell hybrids from each patient are identified by the patient's initials followed by a numerical digit and the suffix "x FO1."

About 5 ml of heparinized blood is used to produce immortalized B cell lines from each patient by incubating PBMC with the supernatant of B95.8 cells (a cell line that chronically produces Epstein-Barr virus) and an anti-CD3 monoclonal antibody (Pharmingen, CA). The B cell lines can then be cryopreserved in 90% FBS/10% DMSO until needed as targets in cytotoxicity experiments designed to test the ability

of patient-derived PBMC to recognize HIV-derived antigenic peptides before and after treatment.

Inclusion Criteria: The following is an example of criteria that may be used to determine whether a patient can be treated with this protocol.

a. any patient age 18 or older with confirmed HIV-1 infection

b. serum creatinine of $\leq$ 1.8 mg/dl

c. total bilirubin of $\leq$ 1.8 mg/dl

d. CD4 count $\geq$ 400/mm3

e. platelet count $\geq$ 100,000/mm^3

f. women of child-bearing age must (have negative pregnancy test and) take adequate precaution to prevent pregnancy during treatment; men should take adequate precaution to prevent pregnancy of partner by abstinence or effective method of birth control

g. documented antiretroviral treatment with at least 3 drugs for at least 6 months prior to enrollment

h. signed informed consent

Exclusion Criteria The following is an example of criteria that may be used to determine whether a patient should not be treated with this protocol.

a. corticosteroid administration within the previous six months

b. AIDS-defining illness, including mucocutaneous Kaposi's sarcoma

c. history of autoimmune disease with potentially life-threatening complications

d. pregnancy or lactation

e. recreational drug/alcohol use that, in the opinion of the principal investigator, would affect patient safety and/or compliance

f. prior medical history of transplantation

g. concurrent malignancy requiring systemic chemotherapy

h. active acute infection

i. use of cytokines (with the exception of erythropoietin and recombinant human growth hormone), systemic corticosteroids, hydroxyurea, immunomodulatory therapy, cytotoxic agents, or antimetabolites

j. clinically significant pulmonary or cardiac disease

k. clinically significant hepatic, hematologic, thyroid, or renal dysfunction

l. any medical or psychiatric condition that would compromise the patient's ability to give informed consent

m. major surgery within 3 weeks prior to enrollment

Pre-treatment Studies The following studies are examples of tests that can be performed prior to the initiation of treatment under this protocol.

a. confirmation of HIV-1 infection

b. complete physical examination to determine if patients meet eligibility/exclusion criteria

c. comprehensive metabolic panel, including as a minimum: electrolytes, creatinine, blood urea nitrogen, glucose, TSH, aspartate amino transferase (AST/SGOT), alanine amino transferase (ALT/SGPT), total bilirubin, creatinine kinase (CK), LDH, calcium, phosphorus, magnesium, and albumen

d. CBC, differential, CD4 count, platelet count

e. measurement of viral load

f. urinalysis

g. skin tests: mumps and candida (recall antigens), $2x10^6$ autologous irradiated PBMC (negative control), $2x10^6$ irradiated semi-allogeneic hybrids (allo)

h. drawing of ~30 ml of blood from patients in order to obtain PBMC to measure *in vitro* cytotoxic response against HIV-derived antigenic peptides and to measure the presence of soluble factors with anti-HIV activity

i. drawing of ~15 ml blood from eligible patients expressing the HLA-A2 antigen, in order to measure *in vitro* cytotoxic response against influenza- and HIV-derived, HLA-A2-restricted peptides

Administration of Semi-Allogeneic Cell Hybrids

At the time of injection, a cryogenic vial containing patient-specific, semi-allogeneic hybrids is thawed to prepare the cells for injection. The cells are gently thawed in 1 ml FBS, washed three times in HBSS, counted, and tested for viability by

trypan blue exclusion (not less than 70% viability will be acceptable). 1.0-3.0x10^7 cell hybrids (depending on appropriate dose) are resuspended in 0.1 ml/1.0x10^7 cells of injectable saline containing a mixture of HIV-derived peptides and loaded in a 1-ml tuberculin syringe. The resulting suspension is exposed to a single lethal dose of 25 Gy γ-rays. Excess numbers of cell hybrids are used in microbiology testing as described below.

Sterility and mycoplasma testing is performed on the final cell hybrid preparation for injection. Specifically, gram stain and microbiology culture are performed by the clinical laboratories. Mycoplasma testing is performed utilizing the PCR-based detection kit manufactured by Stratagene, which allows the identification of any of five species of mycoplasma commonly associated with cell culture infections. Endotoxin testing is performed on the supernatant from the final cell wash using the Limulus Amebocyte Lysate-based kit (Pyrogent Plus Gel-Clot LAL) manufactured by Bio-Whittaker (Walkersville, MD).

The irradiated cell/peptide mixture is administered by intradermal injection at the upper arm and thigh (alternating sides) or as deemed appropriate by the physician.

Two days before the 1st injection, and one and four weeks after the 4th injection, patients are skin tested for DTH response by thawing cryogenic vials of hybrid cells and patient-derived PBMC (as a control). The cells are gently thawed in 1 ml FBS, washed three times in HBSS, counted, and tested for viability by trypan blue exclusion. 2.0x10^6 cells of each type are resuspended in 0.1 ml injectable saline, loaded in 1-ml syringes, irradiated, and intradermally administered into the forearm of the patient, as deemed appropriate by the physician. To appropriately blind the study, syringes are marked A and B, and their identity kept in the laboratory preparing the skin test.

At time of initial screening (approximately eight weeks before potential enrollment in study), two days before the 1st injection, and one and four weeks after

the 4th injection, patients are also skin tested for DTH response by intradermal injection with tests for Candida and mumps recall antigens available through the pharmacy. DTH results are scored by an independent physician 48 hours after skin test placement.

Dose escalation

Patients are enrolled in the study and treated in cohorts of five patients per dose level. The initial dose is $1x10^7$ cell hybrids per injection and is escalated by $1x10^7$ cells per dose up to a maximum of $3x10^7$ cells per injection, which implies the enrollment of fifteen patients divided into three cohorts of five patients each per study site (MUSC and NIH). Therefore, approximately thirty patients are in this study.

Patients in each cohort are assessed for toxicity after the administration of the fourth injection, and if there has been no unexpected toxic reaction, the next dose level (next cohort) is used. Based on the experience of two completed phase I clinical studies, no significant toxicity associated with this study is expected.

Testing of anti-viral cytotoxicity in patients

Immortalized B lymphocytes (derived from each patient) expressing HIV-derived antigenic peptides are used as targets in cytotoxicity experiments designed to determine the ability of patient-derived PBMC to recognize HIV-infected cells. At time of enrollment (day –2 in schema) and one and four weeks after completing treatment, ~30 ml of heparinized whole blood are drawn, and PBMC are obtained by centrifugation through a ficoll gradient (lymphocyte separation medium) as described above.

$3x10^6$ PBMC are incubated for 6 days with $1x10^6$ irradiated (25 Gy) semi-allogeneic hybrids and HIV-derived peptide pool (2.5 μ:M) at 37°C in a humidified 5% CO_2 incubator in RPMI 1640 medium supplemented with 5% human AB^+ serum. The cells are washed and resuspended in RPMI 1640 medium with 10% FBS and used as effectors in a CTL assay. EBV-immortalized B cells expressing HIV-derived

antigenic peptides (target cells) are labeled with ^{51}Cr isotope and pulsed overnight with either HIV peptide pool (5 μ:M) or no peptides. Additional targets are immortalized B cells infected with influenza virus. After three washes, the targets are resuspended at $5x10^3$ cells/well in RPMI 1640 containing 10% FBS and incubated in a 96-well round bottom microtiter plate in triplicate. Stimulated PBMC effector cells are added at Effector:Target ratios 20:1, 10:1, and 5:1. Spontaneous release is determined in target cells cultured in medium alone, and maximum release is determined from targets cultured with 5% Triton X-100. After six hours' exposure, supernatants are analyzed for ^{51}Cr release in a gamma counter. Percent specific lysis is determined as 100 X (experimental cpm - spontaneous cpm)/(maximum cpm -spontaneous cpm).

If patient is HLA-A2+, at time of enrollment (day -2) and one and four weeks after completing treatment, ~15 ml of heparinized whole blood are drawn in order to derive PBMC for in vitro testing of their ability to recognize Flu-M1 and HIVgag-derived, HLA-A2-restricted antigenic peptides. PBMC are obtained by centrifugation through a ficoll gradient (lymphocyte separation medium) as described above. Aliquots of ~$5x10^6$ PBMC are propagated in Iscove's medium supplemented with 10% human AB serum (complete medium) containing 300 IU/ml IL-2 and 5 μ:g/ml of either FLU-$M1_{58-66}$ (GILGFVFTL) (61) or HIVgag_{77-85} (SLYNTVATL) (62) peptides. After 12-14 days of antigenic stimulation, PBMC are used as effector cells in cytotoxicity experiments. As targets, T2 cells (63) are loaded overnight at 26°C with ^{51}Cr isotope and 5 μ:g/ml Flu-M1 or HIVgag77-85 peptides. T2 and Daudi cells load with ^{51}Cr isotope alone will be used as control targets for non-specific lysis. PBMC effectors and ^{51}Cr-loaded target cells will be mixed at 1:1, 5:1, and 15:1 ratios in 96-well plates, and incubated at 37°C in Iscove's complete medium. Spontaneous release is determined in target cells cultured in medium alone, and maximum release is determined from targets cultured with 5% Triton X-100. After four hours exposure, supernatants will be analyzed for ^{51}Cr release in a gamma counter. Percent specific lysis is determined as 100 X (experimental cpm - spontaneous cpm)/(maximum cpm - spontaneous cpm).

Measurement of soluble factors with anti-HIV activity

At time of enrollment (day -2) and one and four weeks after completing treatment, supernatants from the stimulation assay of patient-derived PBMC exposed to semi-allogeneic cell hybrids and the HIV-peptide pool (described above) will be tested for the presence of soluble factors with anti-HIV activity. These experiments will be conducted in laboratories at the NCI according to previously described methods (55, 56).

Off-study criteria

Patients will be taken off study for:

a) voluntary withdrawal or withdrawal of consent

b) inability to comply with treatment schema

c) development of AIDS-related illnesses

d) investigator's discretion

Criteria of biological response

Primary Objectives

Biological response will be determined by comparing DTH response to recall antigens (mumps and Candida) and to allo stimulation (the patient's own semi-allogeneic hybrids) between time of enrollment (time of 1st injection) and time of evaluation (1 and 4 weeks after the 4th injection). DTH response will be read 48 hours after skin test placement and will be measured as the product of two perpendicular diameters (in millimeters) of any induration/erythema exhibited by the patient at each injection site. A positive response will be defined as a restoration or improvement in DTH response to recall antigens and allo stimulation before and after treatment.

PBMCs are added to PHA-stimulated PBMCs during the *in vitro* infection of these PHA-stimulated cells with HIV. This procedure is performed to determine whether the 7-day, hybrid-stimulated culture supernatants contain anti-viral activity that inhibits *in vitro* HIV replication as previously reported (54, 55).

Biological response will also be determined by comparing cytotoxic activity of patient-derived PBMC cells expressing HIV-derived antigenic epitopes, between time of enrollment and time of evaluation (as defined above). A positive response will be defined as the measurable acquisition or improvement in the cytotoxic activity of patient-derived PBMC toward immortalized B lymphocytes (from the same patient) expressing HIV-derived antigenic peptides.

Secondary Objectives

For the secondary objectives of this study, CD4 counts and viral load will be monitored throughout the duration of the study. Also, two days before the 1st injection, and one and four weeks after the 4th injection, patient-derived PBMC will be tested for the ability to generate soluble factors with anti-HIV activity when exposed to semi-allogeneic cell hybrids and the HIV-specific peptide pool.

If patient is HLA-A2+, two days before the 1st injection, and one and four weeks after the 4th injection, their PBMC will also be tested for the ability to recognize specific, HLA-A2-restricted Flu-M1 and HIVgag-derived peptides. For HLA-A2+ patients, a positive response will be defined as the measurable acquisition or improvement in the cytotoxic activity of patient-derived PBMC (after treatment) toward target cells loaded with FLU-M1 and HIVgag-derived peptide.

Concurrent therapy

Concurrent antiretroviral drug treatment for HIV-infection is required for enrollment into the study (see Inclusion Criteria). Other immunotherapeutic/cytokine therapy will not be permitted for the duration of the study.

Anticipated Toxicity

Injection: a local inflammatory reaction would be expected with both the treatment injection and possibly the skin test. It is expected that this reaction will be short-lived and self-limiting.

Autoimmune reactions: Since the treatment contains material derived from autologous cells, there is a possibility of developing autoimmune reactions. In such a case, treatment will be discontinued, and any manifestation of autoimmune disease will be treated accordingly.

Adverse Drug Reactions (ADR)

All adverse events are to be reported by the patient and recorded by the investigator. Events will be evaluated as drug or disease related (or neither), and appropriate action will be taken by the investigator if clinically significant.

EXAMPLE 4

Cytotoxicity studies with PBMC-derived effector cells

Cancer patients generally fail to mount an effective immune response against antigens expressed by their autologous tumor; in other words, they fail to mount a "recall" response to tumor-associated antigens, even though they had been previously "primed" by their autologous tumor. Therefore, a delayed-type hypersensitivity (DTH) response (induration with redness) 48 hours after the intradermal injection of autologous tumor cells is often considered a positive indicator of an immune response developed by the patient against his/her own tumor. In an attempt to establish experimental conditions for the ability of semi-allogeneic cell hybrids to stimulate a recall anti-tumor response by melanoma patients, preliminary studies were conducted on effector cells generated from PBMC obtained from healthy donors, and the HLA-A2-restricted FLU-M1 peptide from the influenza virus was selected as a molecular target (65), since influenza is accepted as a standard immunogen for eliciting a recall immune response in any subject.

White blood cells from leukapheresis of a healthy HLA-$A2^+$ volunteer were separated through ficoll gradients to isolate PBMC, that were in part cryogenically stored and in part used to prepare semi-allogeneic cell hybrids by fusion with FO1-12 cells. Cell hybrids derived from the fusion of FO1-12 cells with PBMC were selected by virtue of their HAT-resistant and neomycin-resistant phenotype by propagation in selective medium for three to four weeks. Immunostaining of drug-resistant cells confirmed that the cell hybrids expressed both HLA class I (HLA-A2) and class II (DR) surface markers.

To generate effector cell populations, PBMC were cultured in Iscove's medium supplemented with 10% human AB serum and interleukin-2 (IL-2, Proleukin, Cetus-Chiron, 300 IU/ml), since lymphocytes cannot be maintained in culture at sufficient numbers without this cytokine.

Briefly, different aliquots of PBMC were exposed to: 1) 1 μg/ml HLA-A2-restricted FLU-M1 peptide (FLU-$M1_{58-66}$ GILGFVFTL) of the influenza virus; 2) 1 μg/ml HLA-A2-restricted FLU-M1 peptide plus irradiated, semi-allogeneic hybrids derived from the fusion of the PBMC with FO1-12 cells; or 3) no antigen, as a negative control. PBMC underwent two cycles of antigenic stimulation for a total of 14 days. PBMC-derived effector cells were then harvested and used in cytotoxicity experiments. Target cells for these assays were T2 cells (66); these cells are commonly used as targets for cytotoxicity experiments because they express empty HLA-A2 class I molecules on their surface and can be easily loaded with exogenous antigenic peptides. T2 cells were loaded with radioactive ^{51}Cr and 5 μg/ml FLU-M1 peptide. Unloaded T2 cells (no peptide) and Daudi cells were used as control targets for non-specific, lymphokine-activated killing. Lysis was performed for four hours with effector:target ratios of 1:1 and 5:1, and the release of radioactivity was measured in a gamma counter. The results of these studies are shown in Table 7.

EFFECTORS	E:T RATIO	TARGET CELLS (% lysis)		
		T2 + FLU	T2 Unloaded	DAUDI
PBMC + FLU	1:1	68	1	0
	5:1	97	0	0
PBMC + FLU + HYB	1:1	52	1	1
	5:1	100	0	0
PBMC alone	1:1	6	5	8
	5:1	15	11	26

Table 7. Cytotoxicity assay showing % lysis (^{51}Cr-release) values of T2 target cells that have been loaded with exogenous FLU-M1 peptide (or unloaded control). Effector cells are PBMC from a healthy HLA-A2$^+$ volunteer that have been exposed to the same peptide with or without irradiated, semi-allogeneic hybrids (HYB) derived from the fusion of the volunteer's PBMC with FO1-12 cells. Daudi cells not loaded with peptide are used as a control for lymphokine-activated killing. Lysis with effector:target ratios of 1:1 and 5:1 are shown.

The results of these cytotoxicity studies suggest that the experimental conditions employed to measure a recall response against FLU are essentially optimal. As expected, the presence of semi-allogeneic hybrids does not interfere with a FLU-specific recall response in healthy volunteers. Moreover, hybrid cell lines formed by the fusion of FO1-12 cells with PBMC from HIV-infected individuals can present HIV-derived ENV peptides to patients' CTL precursors, resulting in the generation of ENV-specific CTL as a measurable indicator of a positive recall immune response.

Consistent with these observations, the results of additional cytotoxicity studies carried out more recently have shown that semi-allogeneic hybrids can also enhance a specific cytotoxic response against HIV-GAG in PBMC obtained from an HLA-A2$^+$, HIV-infected patient; this patient consented to undergo leukapheresis in order to obtain PBMC that were utilized to generate semi-allogeneic hybrids and to derive effector cells for cytotoxicity studies. Specifically, PBMC were propagated for

two weeks in IL-2-containing medium with: A) no antigen; B) irradiated, semi-allogeneic hybrids; C) 1 μg/ml HLA-A2-restricted FLU-M1 peptide; D) 1 μg/ml FLU-M1 peptide plus irradiated, semi-allogeneic hybrids; E) 5 μg/ml HIV-1-derived, HLA-A2-restricted GAG peptide (*gag*77-85, SLYNTVATL) (62); or F) 5 μg/ml GAG peptide plus irradiated, semi-allogeneic hybrids. Target cells for these experiments were: 1) unloaded T2 cells; 2) T2 cells loaded with FLU-M1 peptide; 3) T2 cells loaded with GAG peptide; 4) Daudi cells (Fig. 11). In Fig. 11 results of cytotoxicity assays (^{51}Cr release) showing % lysis values of T2 target cells loaded with HLA-A2-restricted exogenous peptides (FLU-M1 or HIV-1-derived GAG). Unloaded T2 cells and Daudi cells were used as controls. Each panel (A-F) represents PBMC effector cells from an HIV-infected, HLA-$A2^+$ patient that have been exposed to: A) no antigen; B) irradiated, semi-allogeneic hybrids (HYB); C) FLU-M1 peptide; D) FLU-M1 peptide plus irradiated, semi-allogeneic hybrids; E) GAG peptide; or F) GAG peptide plus irradiated, semi-allogeneic hybrids. Lysis with effector:target ratios of 1:1 and 5:1 are shown.

The outcome of these cytotoxicity studies indicates that exposure of the patient's PBMC to FLU peptide results in a measurable cytotoxic response against T2 cells loaded with the same peptide (Fig. 11C), and this response appears to be even stronger when PBMCs are exposed to FLU peptide and irradiated, semi-allogeneic hybrids derived from the patient (Fig. 11D). Interestingly, exposure of the patient's PBMC to HLA-A2-restricted GAG peptide does not appear to result in significant cytotoxicity against T2 cells loaded with GAG peptide (Fig. 11E) unless irradiated, semi-allogeneic hybrids from the patient are also present during the process of antigenic stimulation (Fig. 11F). Because of the ability of irradiated, semi-allogeneic hybrids to restore specific cytotoxicity against HIV-GAG-expressing target cells, it appears that semi-allogeneic hybrids can help overcome immunotolerance to a given antigen.

Throughout this application various publications are referenced by numbers within parentheses. Full citations for these publications are as follows. The

disclosures of these publications in their entireties are hereby incorporated by reference into this application in order to more fully describe the state of the art to which this invention pertains.

REFERENCES

1. Boon, T. Toward a genetic analysis of tumor rejection antigens. Adv Cancer Res, 58: 177-210, 1992.

2. Kawakami, Y, Eliyahu, S, Delgado, CH, Robbins, PF, Rivoltini, L, Topalian, SL, Miki, T, and Rosenberg, SA. Cloning of the gene coding for a shared human melanoma antigen recognized by autologous T cells infiltrating into tumor. Proc Natl Acad Sci USA, 91:3515-3519, 1994.

3. Kawakami, Y, Eliyahu, S, Delgado, CH, Robbins, PF, Sakaguchi, K, Appella, E, Yannelli, JR, Adema, GJ, Miki, T, and Rosenberg, SA. Identification of a human melanoma antigen recognized by tumor-infiltrating lymphocytes associated with *in vivo* tumor rejection. Proc Natl Acad Sci USA, 91:6458-6462, 1994.

4. Brichard, V, Van Pel, A, Wolfel, T, Wolfel, C, De Plaen, E, Lethè, B, Coulie, P, and Boon, T. The tyrosinase gene codes for an antigen recognized by autologous cytolytic T lymphocytes on HLA-A2 melanomas. J Exp Med, 178:489-495, 1993.

5. Chen, L, Ashe, S, Brady, WA, Hellstrom, KE, Ledbetter, JA, McGowan, P, and Linsley, PS. Costimulation of antitumor immunity by the B7 counterreceptor for the T lymphocyte molecules CD28 and CTLA-4. Cell, 71: 1093-1102, 1992.

6. Townsend, SE and Allison, JP. Tumor rejection after direct costimulation of CD8+ T cells by B7-transfected melanoma cells. Science, 259: 368-370, 1993.

7. Plautz, GE, Yang, ZY, Wu, BY, Gao, X, Huang, L, and Nabel, GJ. Immunotherapy of malignancy by in vivo gene transfer into tumors. Proc Natl Acad Sci USA, 90: 4645-4649, 1993.

8. Jami, J and Ritz, E. Expression of tumor-specific antigens in mouse somatic cell hybrids. Cancer Res, 33: 2524-2528, 1973.

9. Parkman, R. Tumor cell hybrids: an immunotherapeutic agent. J Natl Cancer Inst, 52: 1541-1545, 1974.

10. Kim, BS, Liang, W, and Cohen, EP. Tumor-specific immunity induced by somatic hybrids. I. Lack of relationship between immunogenicity and tumorigenicity of selected hybrids. J Immunol, 123: 733-738, 1979.

11. Kim, BS. Tumor-specific immunity induced by somatic hybrids. II. Elicitation of enhanced immunity against the parent plasmocytoma. J Immunol, 123: 739-744, 1979.

12. Payelle, B, Poupon, M-F, and Lespinats, G. Adoptive transfer of immunity induced by semi-allogeneic hybrid cells, against a murine fibrosarcoma. Int J Cancer, 27: 783-788, 1981.

13. Toffaletti, DL, Darrow, TL, and Scott, DW. Augmentation of syngeneic tumor-specific immunity by semi-allogeneic cell hybrids. J Immunol, 130: 2982-2986, 1983.

14. Henderson, RA, Finn, OJ. Human tumor antigens are ready to fly. Adv Immunol, 62:217-256, 1996.

15. Berzofsky, JA, and Berkover, IJ. Novel approaches to peptide and engineered protein vaccines for HIV using defined epitopes: advances in 1994-1995. AIDS 9 (suppl. A):S143-S157, 1995.

16. Levy, J.P. Questions about CD8+ anti-HIV lymphocytes in the control of HIV infection. Antibiot Chemother 48:13-20, 1996.

17. Giovanella, BC, Stehlin, JS, Santamaria, C, Yim, SO, Morgan, AC, Williams, LJ, Leibovitz, A, Fialkow, PJ, and Mumford, DM. Human neoplastic and normal cells in tissue culture. I. Cell lines derived from malignant melanomas and normal melanocytes. J Natl Cancer Inst, 56: 1131-1142, 1976.

18. D'Urso, CM, Wang, Z, Cao, Y, Tatake, R, Zeff, RA, and Ferrone, S. Lack of HLA class I antigen expression by cultured melanoma cells FO-1 due to a defect in β_2m gene expression. J Clin Invest, 87:284-292, 1991.

19. Kageshita, T, Wang, Z, Calorini, L, Yoshi, A, Kimura, T, Ono, T, Gattoni-Celli, S, and Ferrone, S. Selective loss of human leukocyte class I allospecificities and staining of melanoma cells by monoclonal antibodies recognizing monomorphic determinants of human leukocyte class I antigens. Cancer Res, 53:3349-3354, 1993.

20. Wigler, M, Pellicer, A, Silverstein, S, and Axel, R. Biochemical transfer of single copy eukaryotic genes using total cellular DNA as donor. Cell, 14:725-731, 1978.

21. Gorman, C, Padmanabhan, R, and Howard, BH. High efficiency DNA-mediated transformation of primate cells. Science, 221: 551-553, 1983.

22. Pontecorvo, G. Production of mammalian somatic cell hybrids by means of polyethylene glycol treatment. Somatic Cell Genet. 1: 397-400, 1975.

23. Prado, AA, Partearroyo, MA, Mencia, M, Goni, FM, and Barbera-Guillem, E. Surfactant enhancement of polyethyleneglycol-induced cell fusion. FEBS Lett, 259: 149-152, 1989.

24. Galfre, G, Howe, SC, Milstein, C, Butcher, GW, and Howard, JC. Antibodies to major histocompatibility antigens produced by hybrid cell lines. Nature, 266: 550-552, 1977.

25. Yannelli, JR, Hyatt, C, McConnell, S, Hines, K, Jacknin, L, Parker, L, Sanders, M, and Rosenberg, SA. The growth of tumor-infiltrating lymphocytes from human solid cancers: summary of a 5-year experience. Int J Cancer, 65: 413-421, 1996.

26. Clerici, M, Lucey, DR, Zajac, RA, Boswell, RN, Gebel, HM, Takahashif, H, Berzofsky, JA, and Shearer, GM. Detection of cytotoxic T lymphocytes specific for synthetic peptides of gp160 in HIV-seropositive individuals. J Immunol, 146: 2214-2219, 1991.

27. Shearer GM, Pinto LA, Clerici M: Alloimmunization for immune-based therapy and vaccine design against HIV/AIDS. Immunol Today 1999, 20:66-71.

28. Davey, RT Jr, Bhat N, Yoder C, et al.: HIV-1 and T cell dynamics after interruption of highly active antiretroviral therapy HAART) in patients with a history of sustained viral suppression. Proc Natl Acad Sci USA 1999, 69:15109-15114.

29. Clerici M, Stocks NI, Zajac RA, et al.: Detection of three distinct patterns of T helper cell dysfunction in asymptomatic, human immunodeficiency virus-seropositive patients. Independence of CD4+ cell numbers and clinical staging. J Clin Invest 1989, 84:1892-1899.

30. Clerici M, Stocks NI, Zajac RA, Boswell RN, Via CS, Shearer GM: Circumvention of defective CD4 T helper cell function in HIV-infected individuals by stimulation with HLA alloantigens. J Immunol 1990, 144:3266-3271.

31. Shearer GM: HIV-induced immunopathogenesis. Immunity 1998, 9:587-593.

32. Keene JA, Forman J: Helper activity is required for the in vivo generation of cytotoxic T lymphocytes. J Exp Med 1982, 155:768-782.

33. Shirai M, Pendleton CD, Ahlers J, Takeshita T, Newman M, Berzofsky JA: Helper-CTL determinant linkage required for priming of anti-HIV CD8+ CTL in vivo with peptide vaccine constructs. J Immunol 1994, 152:549-556.

34. Ahlers JD, Takeshita T, Pendleton CD, Berzofsky JA: Enhanced immunogenicity of HIV-1 vaccine construct by modification of the native peptide sequence. Proc Natl Acad Sci USA 1997, 94:10856-10861.

35. Newton DA, McClay E F, Romano C, Gattoni-Celli S: Irradiated semi-allogeneic cell hybrids as therapeutic vaccines for cancer. J Immunother (in press).

36. Cease KB, Margalit H, Cornette JL, et al.: Helper T-cell antigenic site identification in the acquired immunodeficiency syndrome virus gp120 envelope protein and induction of immunity in mice to the native protein using 16-residue synthetic peptide. Proc Natl Acad Sci USA 1987, 84:4249-4253.

37. Takahashi HJ, Cohen J, Hosmalin A, et al.: An immunodominant epitope of the human immunodeficiency virus envelope glycoprotein gp160 recognized by class I major histocompatibility complex molecule-restricted murine cytotoxic T lymphocytes. Proc Natl Acad Sci USA 1988, 85:3105-3109.

38. Hale PM, Cease KB, Houghten RA, et al.: T cell multideterminal regions in the human immunodeficiency virus envelope: towards overcoming the problem of major histocompatibility complex restriction. Int Immunol 1989, 1:409-415.

39. Clerici M, Stocks NI, Zajac RA, et al.: Antigenic peptides recognized by T helper lymphocytes from asymptomatic, HIV seropositive individuals. Nature 1989, 339:383-385.

40. Pinto LA, Berzofsky JA, Fowke KR, et al.: HIV-specific immunity following immunization with HIV synthetic envelope peptides in asymptomatic HIV-infected patients. AIDS 1999, 13:2003-2012.

41. Abdel-Wahab Z, Dar MM, Hester D, et al.: Effect of irradiation on cytokine production, MHC antigen expression, and vaccine potential of interleukin-2 and interferon-gamma gene-modified melanoma cells. Cell Immunol 1996, 171:246-254.

42. Sun Y, Jurgovsky K, Moller P, et al.: Vaccination with IL-12 gene-modified autologous melanoma cells: preclinical results and a first clinical phase I study. Gene Ther 1998, 5:481-490.

43. Rosenberg ES, Billingsley JM, Caliendo AM, Boswell SL, Sax PE, Walker BD: Vigorous HIV-1-specific CD4+ T cell responses associated with control of viremia. Science 1997, 278:1447-1450.

44. Shirai M, Chen M, Arichi T, et al.: Use of intrinsic and extrinsic helper epitopes for in vitro induction of anti-Hepatitis C virus cytotoxic T lymphocytes (CTL) with CTL epitope peptide vaccines. J Infect Dis 1996, 173:24-31.

45. Dudhane A, Conti B, Orlikowsky T, et al.:Monocytes in HIV type 1-infected individuals lose expression of costimulatory B7 molecules and acquire cytotoxic activity. Aids Res Hum Retroviruses 1996, 12:885-892.

46. Moriuchi H, Moriuchi M, Fauci AS: Induction of HIV-1 replication by allogeneic stimulation. J Immunol 1999, 162:7543-7548.

47. Fowke KR, D'Amico R, Chernoff DN, et al.: Immunologic and virologic evaluation after influenza vaccination of HIV-1-infected patients. AIDS 1997, 11:1013-1021.

48. Shearer GM, Pinto LA, Clerici M. Alloimmunization for immune-based therapy and vaccine design against HIV/AIDS. Immunol Today 1999; 20:66-71.

49. Stott EJ. Anti-cell antibody in macaques. Nature 1991; 353:393.

50. Arthur LO, Bess JW Jr, Sowder RC, Benveniste RE, Mann DL, Chermann JC, Henderson LE. Cellular proteins bound to immunodeficiency viruses: implications for pathogenesis and vacines. Science 1992; 258:1935-8.

51. Arthur LO, Bess JW Jr, Urban RG, Strominger JL, Morton WR, Mann DL, Henderson LE, Benveniste RE. Macaques immunized with HLA-DR are protected from challenge with simian immunodeficiency virus. J Virol 1995; 69:3117-24.

52. MacDonald KS, Embree J, Njenga S, Nagelkerke NJ, Ngatia I, Mohammed Z, Barber BH, Ndinya-Achola J, Bwayo J, Plummer FA. Mother-child class I HLA concordance increases perinatal human immunodeficiency virus type 1 transmission. J Infect Dis 1998; 177:551-6.

53. Brühl P, Kerschbaum A, Zimmermann K, Eibl MM, Mannhalter JW. Allostimulated lymphocytes inhibit replication of HIV type 1. AIDS Res Hum Retroviruses 1996; 12:31-7.

54. Pinto LA, Sharpe S, Cohen DI, Shearer GM. Alloantigen-stimulated anti-HIV activity. Blood 1998; 92:3346-54.

55. Pinto LA, Blazevic V, Patterson BK, Dolan MJ, Shearer GM. Alloantigen-induced anti-HIV activity occurs prior to reverse transcription and can be generated by leukocytes from HIV-infected individuals. Blood, 2000; 95: 1875-6.

56. Wang YF, Tao L, Mitchell E, Bravery C, Berlingieri P, Armstrong P, Vaughan R, Underwood J, Lehner T. Allo-immunization elicits $CD8^+$ T cell-derived chemokines, HIV suppressor factors and resistance to HIV infection in women. Nature Med 1999; 5:1004-9.

57. Landay AL, Shearer GM. Immune restoration in HIV disease: a basic immunology perspective. Lancet; in press.

58. Grene E, Newton DA, Brown EA, Berzofsky JA, Gattoni-Celli S, Shearer GM. Semi-allogeneic cell hybrids stimulate HIV-1 envelope-specific cytotoxic T lymphocytes. 1999; submitted for publication.

59. Galfre G, Howe SC, Milstein C, Butcher GW, Howard JC. Antibodies to major histocompatibility antigens produced by hybrid cell lines. Nature 1977; 266:550-2.

60. Prado AA, Partearroyo MA, Mencia M, Goni FM, Barbera-Guillem E. Surfactant enhancement of polyethylene glycol-induced cell fusion. FEBS Lett 1989; 259:149-52.

61. Kim CJ, Prevette T, Cormier J, Overwijk W, Roden M, Restifo N, Rosenberg SA, Marincola FM. Dendritic cells infected with poxviruses encoding MART-1/Melan A sensitive T lymphocytes in vitro. J Immunother 1997; 20:276-86.

62. Tsomides TJ, Aldovini A, Johnon RP, Walker BD, Young RA, Eisen HN. Naturally processed viral peptides recognized by cytotoxic T lymphocytes on cells chronically infected by human immunodeficiency virus type 1. J Exp Med 1994; 180:1283-93.

63. Salter RD, Cresswell P. Impaired assembly and transport of HLA-A and -B antigens in a mutant TxB cell hybrid. EMBO J 1986; 5:943-9.

64. *The Oncogene Handbook*, T. Curran, E.P. Reddy, and A. Salka (ed.), Elsevier Science Publishers, The Netherlands (1988).

65. Bodmer H, Ogg G, Gotch F, McMichael A. Anti-HLA-A2 antibody-enhancement of peptide association with HLA-A2 as detected by cytotoxic T lymphocytes. *Nature* 189; 342:443-6.

66. Salter RD, Cresswell P. Impaired assembly and transport of HLA-A and -B antigens in a mutant TxB cell hybrid. *EmBO J.* 1986; 5:943-9.

What is claimed is:

1. A composition comprising a semi-allogeneic hybrid fusion cell and an immunogenic peptide.

2. The composition of claim 1, wherein the immunogenic peptide is a virus-specific peptide.

3. The composition of claim 1, wherein the immunogenic peptide is a cancer-specific peptide.

4. The composition of claim 1, wherein the hybrid fusion cell is a fusion of a cell that is deficient in β_2 microglobulin, resistant to neomycin and sensitive to HAT and a cell from a human subject.

5. The composition of claim 4, wherein the cell from the human subject is a white blood cell.

6. The composition of claim 1, wherein the hybrid fusion cell is a fusion of a cell from the cell line deposited with the American Type Culture Collection under accession number ATCC CRL-12177 and a white blood cell from a human subject.

7. The composition of claim 2, wherein the hybrid fusion cell presents a virus-specific immunogenic peptide.

8. The composition of claim 7, wherein the virus-specific immunogenic peptide is an HIV peptide

9. The composition of claim 8, wherein the HIV peptide is an *env* peptide.

10. The composition of claim 9, wherein the *env* peptide is selected from the group consisting of *env* T1, *env* T2, *env* Th4.1, *env* P18 and *env* P18 MN.

11. The composition of claim 9, comprising at least two peptides selected from the group consisting of *env* T1, *env* T2, *env* Th4.1, *env* P18 and *env* P18 MN.

12. The composition of claim 8, wherein the HIV-specific peptide is selected from the group consisting of nef, gag and Tat.

13. The composition of claim 12, comprising at least two peptides selected from the group consisting of nef, gag and Tat.

14. A composition comprising isolated *env* T1, isolated *env* T2, isolated *env* Th4.1, isolated *env* P18 and isolated *env* P18 MN.

15. A composition comprising isolated nef, isolated gag and isolated Tat.

16. A composition comprising isolated *env* T1, isolated *env* T2, isolated *env* Th4.1, isolated *env* P18, isolated *env* P18 MN, isolated nef, isolated gag and isolated Tat.

17. A composition comprising at least two isolated peptides selected from the group consisting of isolated *env* T1, isolated *env* T2, isolated *env* Th4.1, isolated *env* P18, isolated *env* P18 MN, isolated nef, isolated gag and isolated Tat.

18. A method of enhancing the cytotoxic T cell activity of a population of peripheral blood mononuclear cells of a human subject, comprising contacting the peripheral blood mononuclear cells of the subject with the composition of claim 1.

19. A method of enhancing the cytotoxic T cell activity of a population of peripheral blood mononuclear cells of a human subject, comprising contacting the peripheral blood mononuclear cells of the subject with the composition of claim 2.

20. A composition comprising a population of PBMCs enriched for cytotoxic T cells produced by the method of claim 18.

21. A composition comprising a population of PBMCs enriched for cytotoxic T cells produced by the method of claim 19.

22. A method of treating an HIV infection in a subject, comprising administering to the subject an effective amount of an isolated HIV-specific peptide in a pharmaceutically acceptable carrier and an effective amount of a hybrid fusion cell in a pharmaceutically acceptable carrier, wherein the hybrid fusion cell is a fusion of a cell that is deficient in β_2 microglobulin, resistant to neomycin and sensitive to HAT and a white blood cell from the subject.

23. The method of claim 22, wherein the isolated HIV-specific peptide and the hybrid fusion cell are administered concurrently.

24. The method of claim 23, wherein at least one isolated HIV-specific peptide is selected from the group consisting of *env* T1, *env* T2, *env* Th4.1, *env* P18 and *env* P18 MN, nef, gag and Tat.

25. The composition of claim 2, wherein the isolated virus-specific immunogenic peptide is selected from the group consisting of HTLV-1, Hepatitis B virus, Hepatitis C virus, rubeola virus, influenza A virus and Human Papilloma Virus.

26. The composition of claim 25, wherein the hybrid fusion cell is a fusion of a cell that is deficient in β_2 microglobulin, resistant to neomycin and sensitive to HAT and a white blood cell from a subject infected with a virus selected from the group consisting of HTLV-1, Hepatitis B virus, Hepatitis C virus, rubeola virus, influenza A virus and Human Papilloma Virus.

27. The composition of claim 25, wherein the hybrid fusion cell is a fusion of a cell from the cell line deposited with the American Type Culture Collection under accession number ATCC CRL-12177 and a white blood cell derived from a subject infected with a virus selected from the group consisting of HTLV-1, Hepatitis B virus, Hepatitis C virus, rubeola virus, influenza A virus and Human Papilloma Virus.

28. The composition of claim 25, wherein the hybrid fusion cell presents a virus-specific immunogenic peptide selected from the group of isolated peptides of HTLV-1, Hepatitis B virus, Hepatitis C virus, rubeola virus, influenza A virus and Human Papilloma Virus.

29. The composition of claim 25, comprising at least two isolated peptides selected from the group consisting of a peptide of HTLV-1, a peptide of Hepatitis B virus, a peptide of Hepatitis C virus, a peptide of rubeola virus, a peptide of influenza A virus and a peptide of Human Papilloma Virus.

30. A composition comprising isolated immunogenic peptides of HTLV-1, Hepatitis B virus, Hepatitis C virus, rubeola virus, influenza A virus and Human Papilloma Virus.

31. A method of enhancing the cytotoxic T cell activity of a population of peripheral blood mononuclear cells of a subject infected with at least one virus selected from the group consisting of HTLV-1, Hepatitis B virus, Hepatitis C virus, rubeola virus, influenza A virus and Human Papilloma Virus, comprising contacting the peripheral blood mononuclear cells of the subject with the composition of claim 25.

32. A composition comprising a population of peripheral blood mononuclear cells enriched for cytotoxic T cells produced by the method of claim 31.

33. A method of treating a subject infected with at least one virus selected from the group consisting of HTLV-1, Hepatitis B virus, Hepatitis C virus, rubeola virus,

influenza A virus and Human Papilloma Virus, comprising administering to the subject an effective amount of the composition of claim 25 in a pharmaceutically acceptable carrier.

34. The method of claim 33, wherein at least one isolated virus-specific immunogenic peptide is selected from the group consisting of a peptide of HTLV-1, a peptide of Hepatitis B virus, a peptide of Hepatitis C virus, a peptide of rubeola virus, a peptide of influenza A virus and a peptide of Human Papilloma Virus.

35. A method of treating a subject infected with at least one virus selected from the group consisting of HTLV-1, Hepatitis B virus, Hepatitis C virus, rubeola virus, influenza A virus and Human Papilloma Virus, comprising administering to the subject an effective amount of a isolated virus-specific peptide selected from the group consisting of a peptide of HTLV-1, a peptide of Hepatitis B virus, a peptide of Hepatitis C virus, a peptide of rubeola virus, a peptide of influenza A virus and a peptide of Human Papilloma Virus in a pharmaceutically acceptable carrier and an effective amount of a hybrid fusion cell in a pharmaceutically acceptable carrier wherein the hybrid fusion cell is a fusion of a cell that is deficient in β_2 microglobulin, resistant to neomycin and sensitive to HAT and a white blood cell derived from the subject infected by a virus.

36. The method of claim 35, wherein the isolated virus-specific peptide and the hybrid fusion cell are administered concurrently.

37. The method of claim 35, wherein at least one isolated virus-specific immunogenic peptide is selected from the group consisting of a peptide of HTLV-1, a peptide of Hepatitis B virus, a peptide of Hepatitis C virus, a peptide of rubeola virus, a peptide of influenza A virus and a peptide of Human Papilloma Virus.

38. The composition of claim 3, wherein the hybrid fusion cell presents an isolated cancer-specific immunogenic peptide.

39. The composition of claim 39, wherein the isolated cancer-specific peptide is specific to a cancer selected from the group consisting of B cell lymphoma, T cell lymphoma, myeloma, leukemia, breast cancer, pancreatic cancer, colon cancer, lung cancer, renal cancer, liver cancer, prostate cancer, melanoma and cervical cancer.

40. A composition comprising isolated cancer-specific immunogenic peptides specific to a cancer selected from the group consisting of B cell lymphoma, T cell lymphoma, myeloma, leukemia, breast cancer, pancreatic cancer, colon cancer, lung cancer, renal cancer, liver cancer, prostate cancer, melanoma and cervical cancer in a pharmaceutically acceptable carrier.

41. A method of enhancing the cytotoxic T cell activity of a population of peripheral blood mononuclear cells of a human subject, comprising contacting the peripheral blood mononuclear cells of the subject with the composition of claim 3.

42. A composition comprising a population of PBMCs enriched for cytotoxic T cells produced by the method of claim 41 in a pharmaceutically acceptable carrier.

43. A method of treating cancer in a subject, comprising administering to the subject an effective amount of a cancer-specific immunogenic peptide in a pharmaceutically acceptable carrier and an effective amount of a hybrid fusion cell in a pharmaceutically acceptable carrier, wherein the hybrid fusion cell is a fusion of a cell that is deficient in β_2 microglobulin, resistant to neomycin and sensitive to HAT and a white blood cell from the subject.

44. The method of claim 43, wherein the isolated immunogenic cancer-specific peptide and the hybrid fusion cell are administered concurrently.

45. The method of claim 44, wherein the isolated immunogenic cancer-specific peptide is specific to a cancer selected from the group consisting of B cell lymphoma,

T cell lymphoma, myeloma, leukemia, breast cancer, pancreatic cancer, colon cancer, lung cancer, renal cancer, liver cancer, prostate cancer, melanoma and cervical cancer.

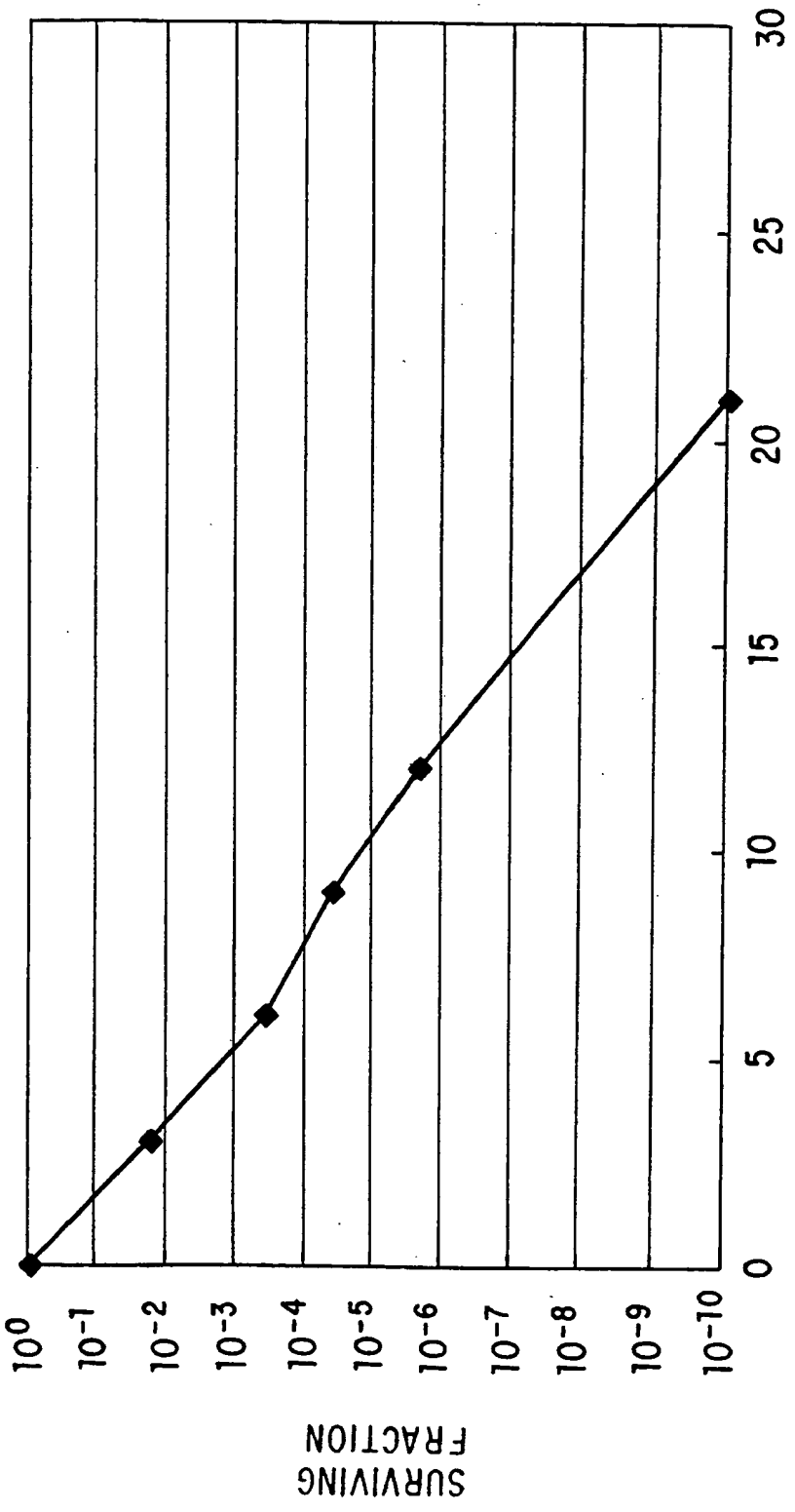

SURVIVING FRACTION
10^{0}
10^{-1}
10^{-2}
10^{-3}
10^{-4}
10^{-5}
10^{-6}
10^{-7}
10^{-8}
10^{-9}
10^{-10}
0
5
10
15
20
25
30
DOSE (Gy)

FIG. 1

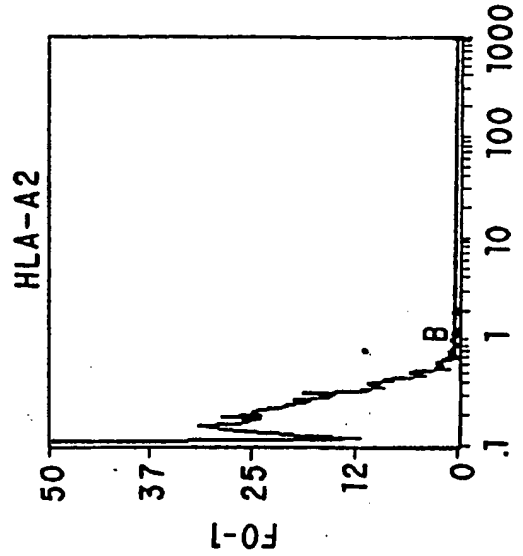


FIG. 2C

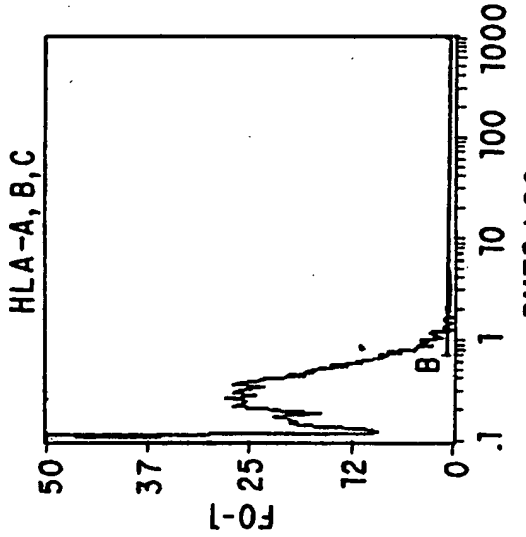


FIG. 2B

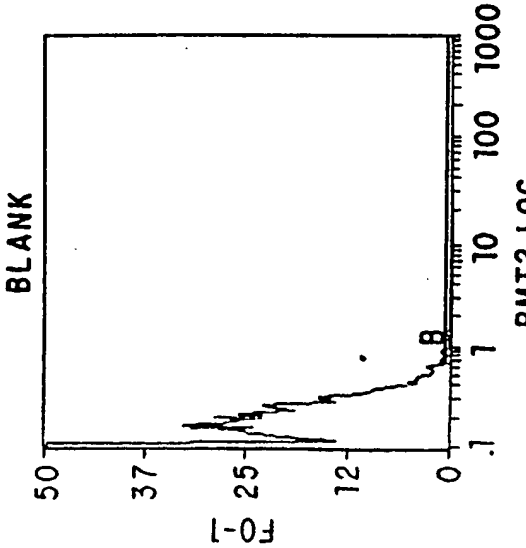


FIG. 2A

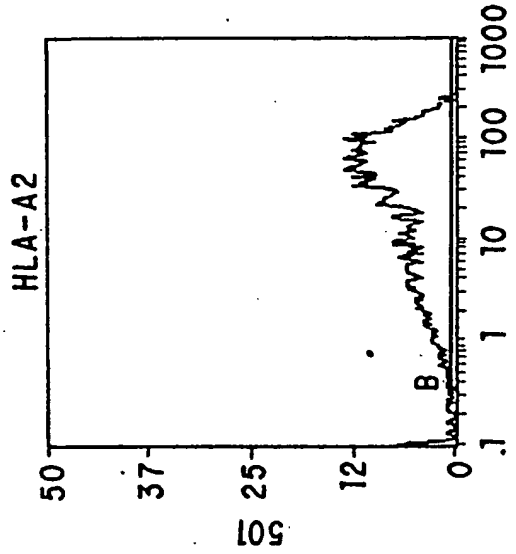


FIG. 2F

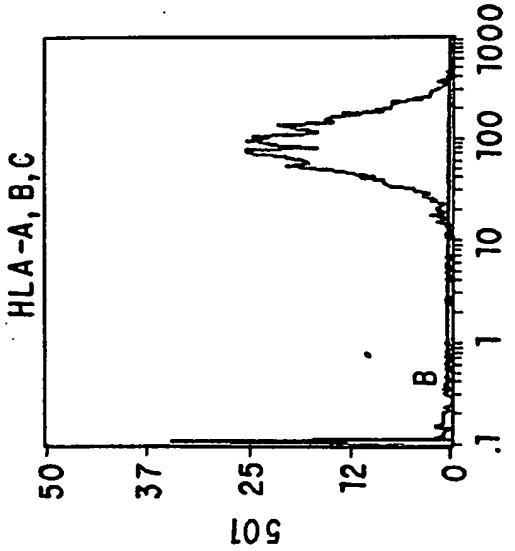


FIG. 2E

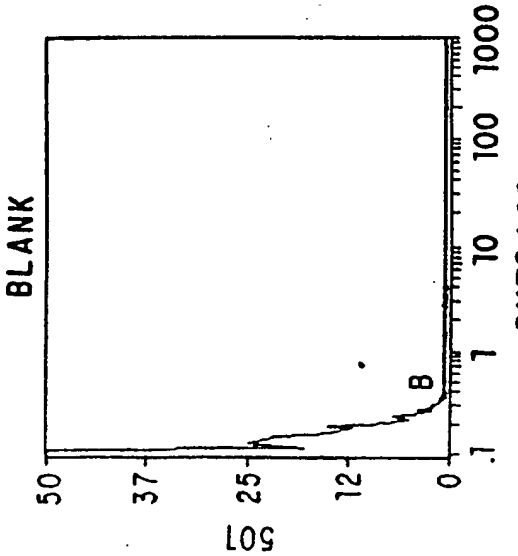


FIG. 2D

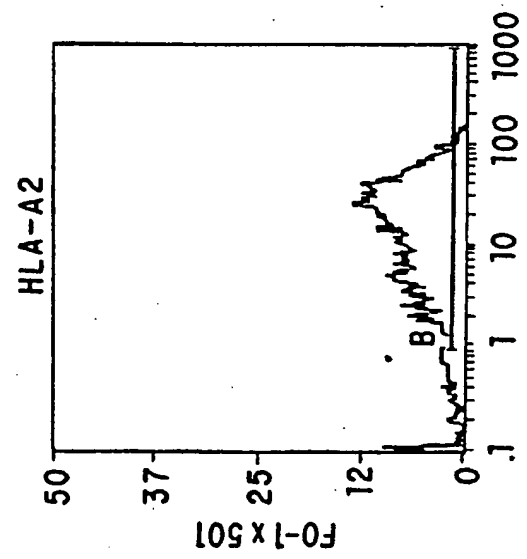


FIG. 2I

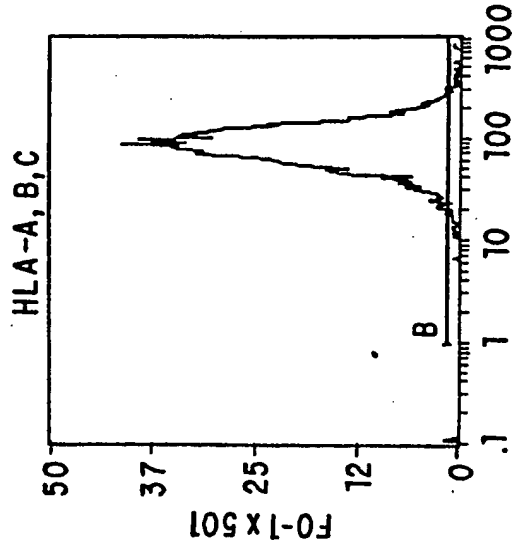


FIG. 2H

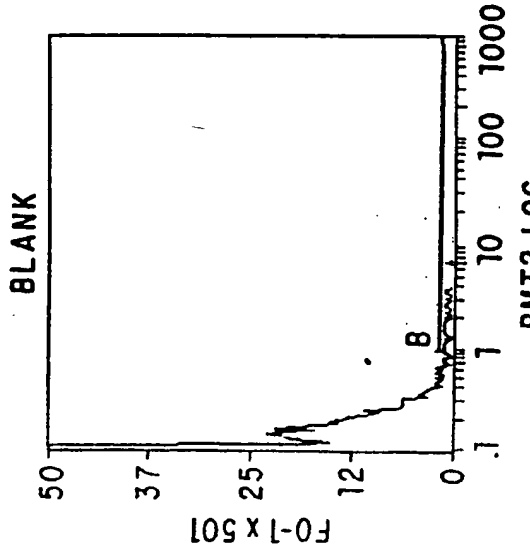


FIG. 2G

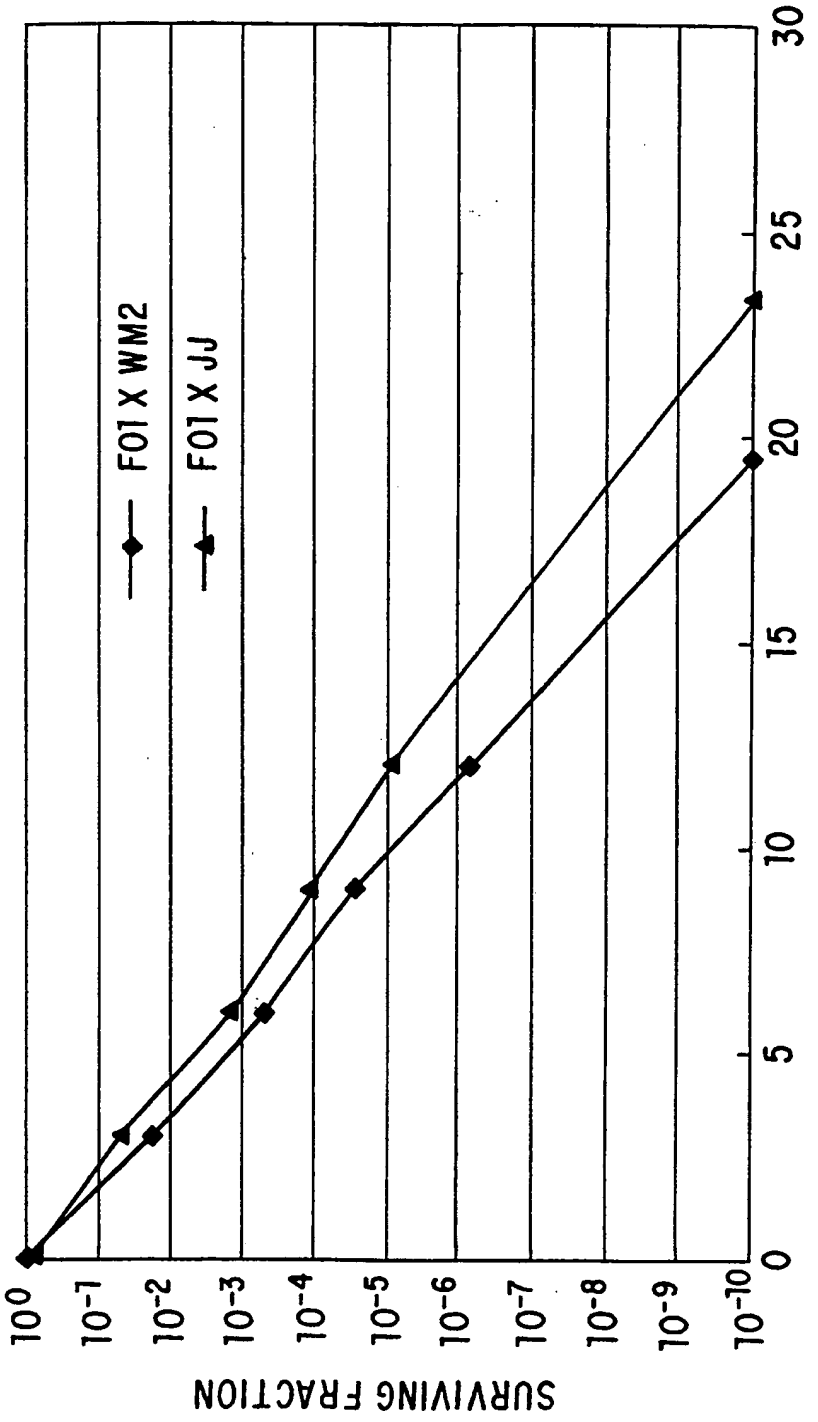
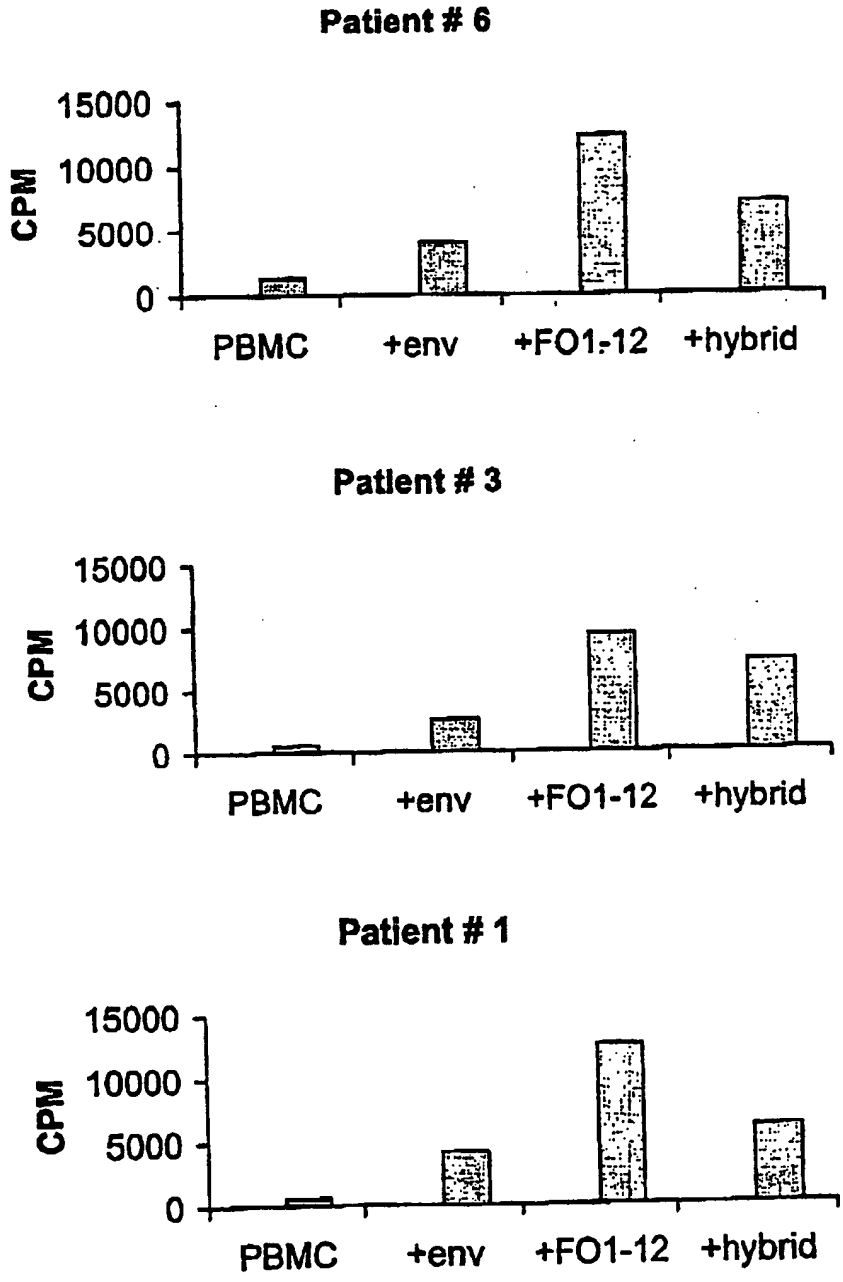

SURVIVING FRACTION
10^{0}
10^{-1}
10^{-2}
10^{-3}
10^{-4}
10^{-5}
10^{-6}
10^{-7}
10^{-8}
10^{-9}
10^{-10}
0
5
10
15
20
25
30
DOSE (Gy)
F01 X WM2
F01 X JJ

FIG. 3

FIG. 4


Patient # 6
15000
10000
5000
0
CPM
PBMC
+env
+FO1-12
+hybrid
Patient # 3
15000
10000
5000
0
CPM
PBMC
+env
+FO1-12
+hybrid
Patient # 1
15000
10000
5000
0
CPM
PBMC
+env
+FO1-12
+hybrid

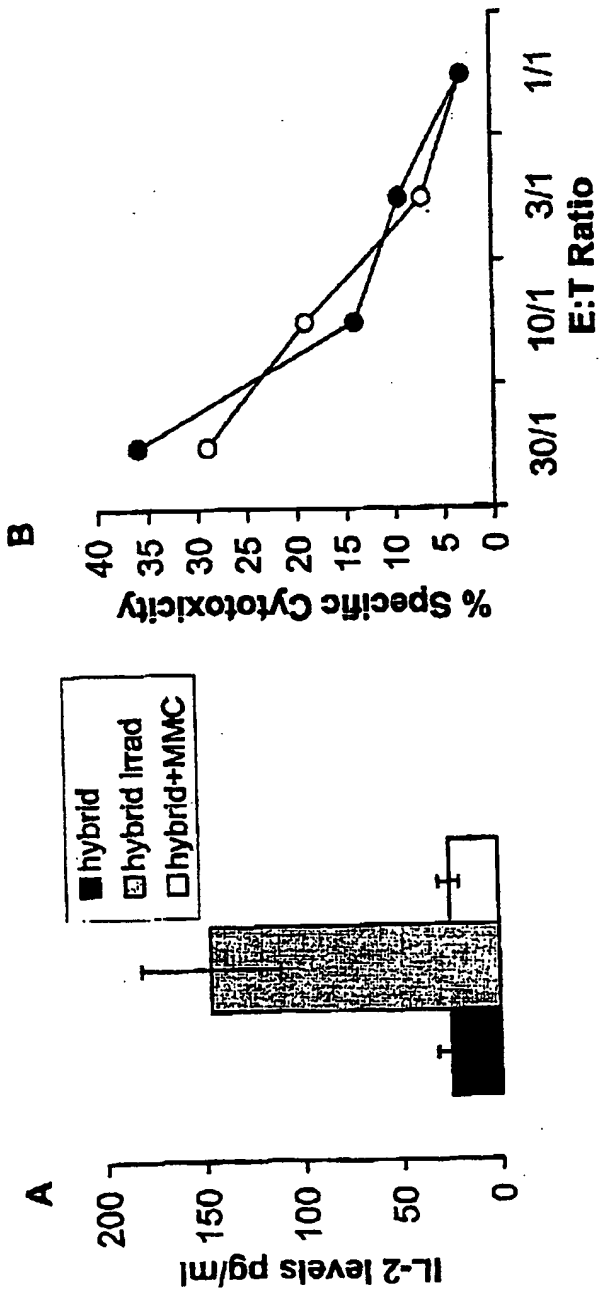

A
IL-2 levels pg/ml
200
150
100
50
0
hybrid
hybrid Irrad
hybrid+MMC
B
% Specific Cytotoxicity
40
35
30
25
20
15
10
5
0
30/1
10/1
3/1
1/1
E:T Ratio

FIG. 5

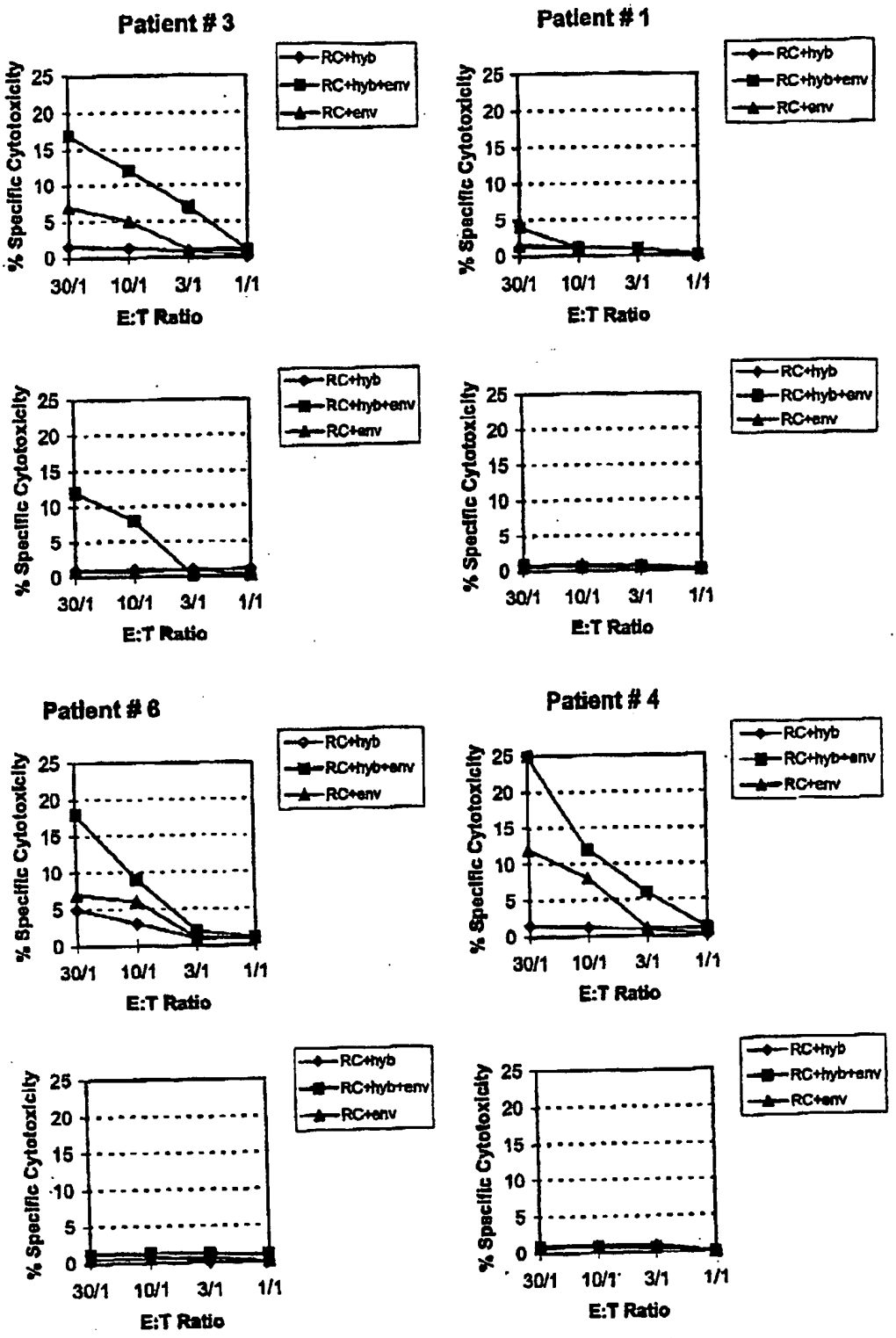

Patient # 3
Patient # 1
Patient # 6
Patient # 4
RC+hyb
RC+hyb+env
RC+env
% Specific Cytotoxicity
0
5
10
15
20
25
30/1
10/1
3/1
1/1
E:T Ratio

FIG. 6

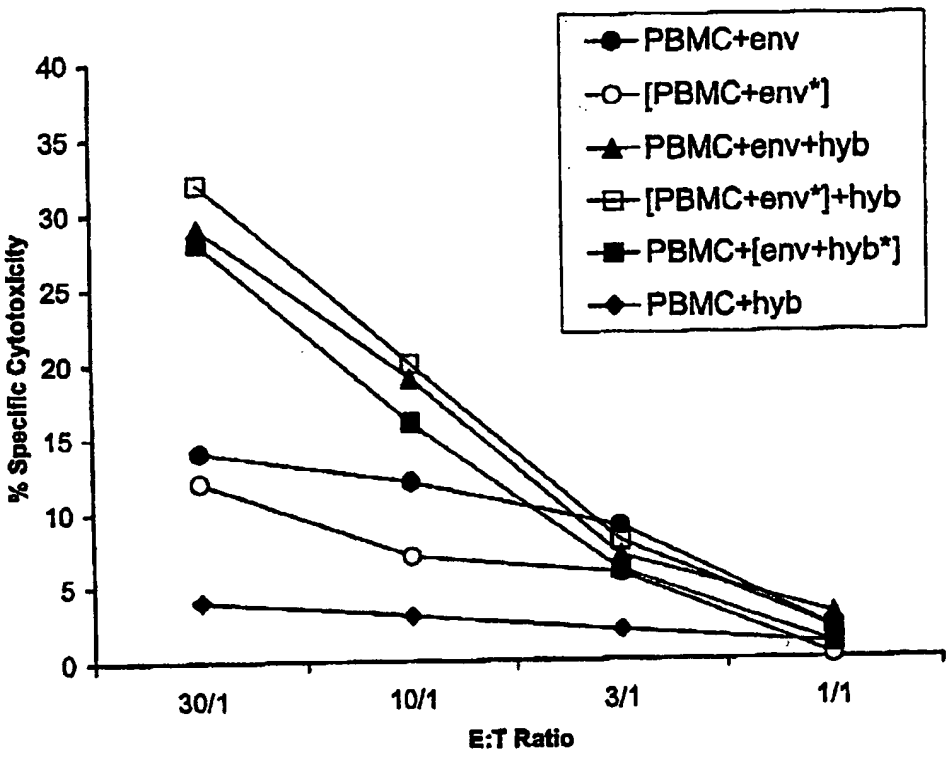

PBMC+env
[PBMC+env*]
PBMC+env+hyb
[PBMC+env*]+hyb
PBMC+[env+hyb*]
PBMC+hyb
% Specific Cytotoxicity
40
35
30
25
20
15
10
5
0
30/1
10/1
3/1
1/1
E:T Ratio

FIG. 7

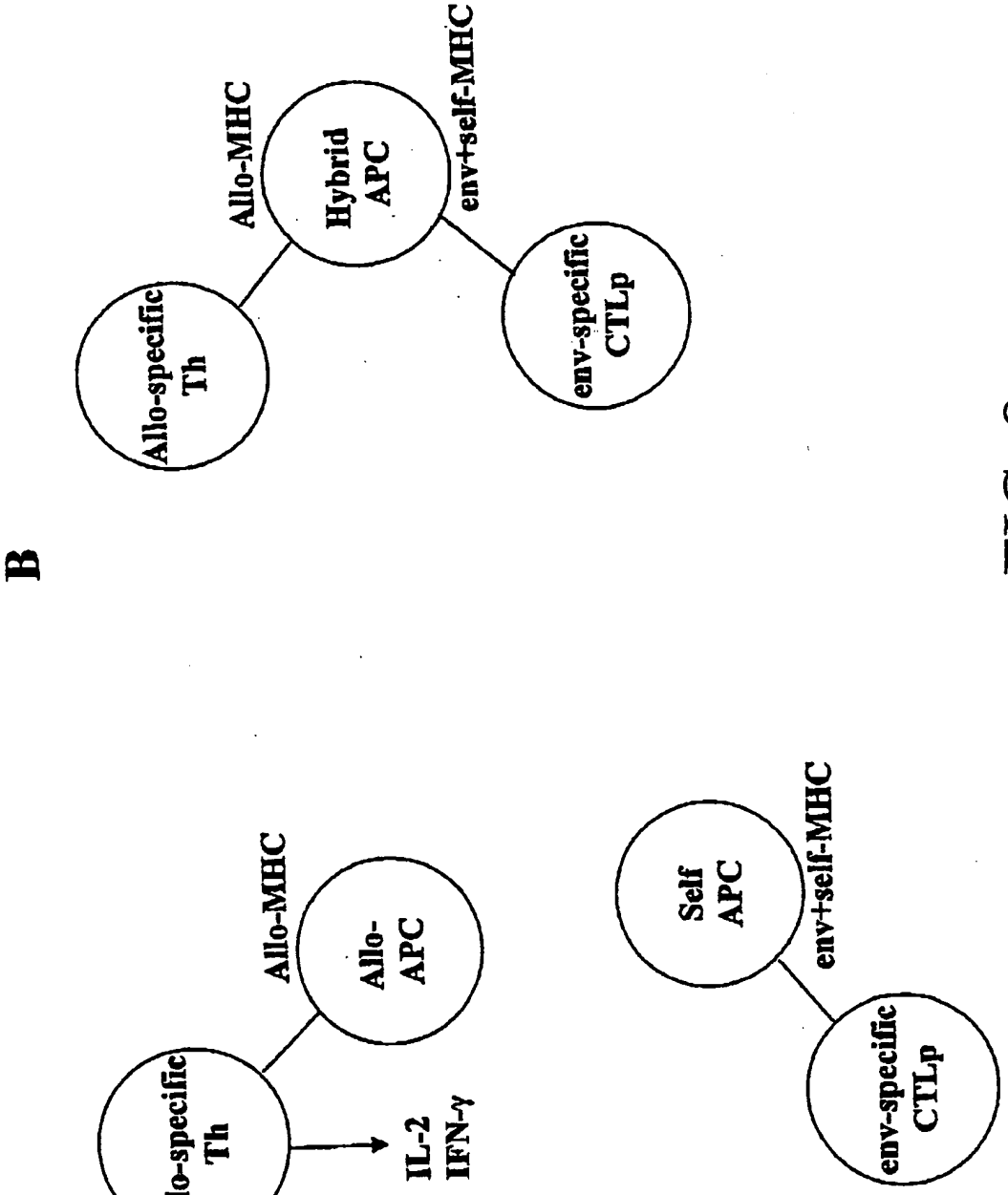

A
Allo-specific Th
Allo-MHC
Allo-APC
IL-2
IFN-γ
Self APC
env+self-MHC
env-specific CTLp
B
Allo-specific Th
Allo-MHC
Hybrid APC
env+self-MHC
env-specific CTLp

FIG. 8

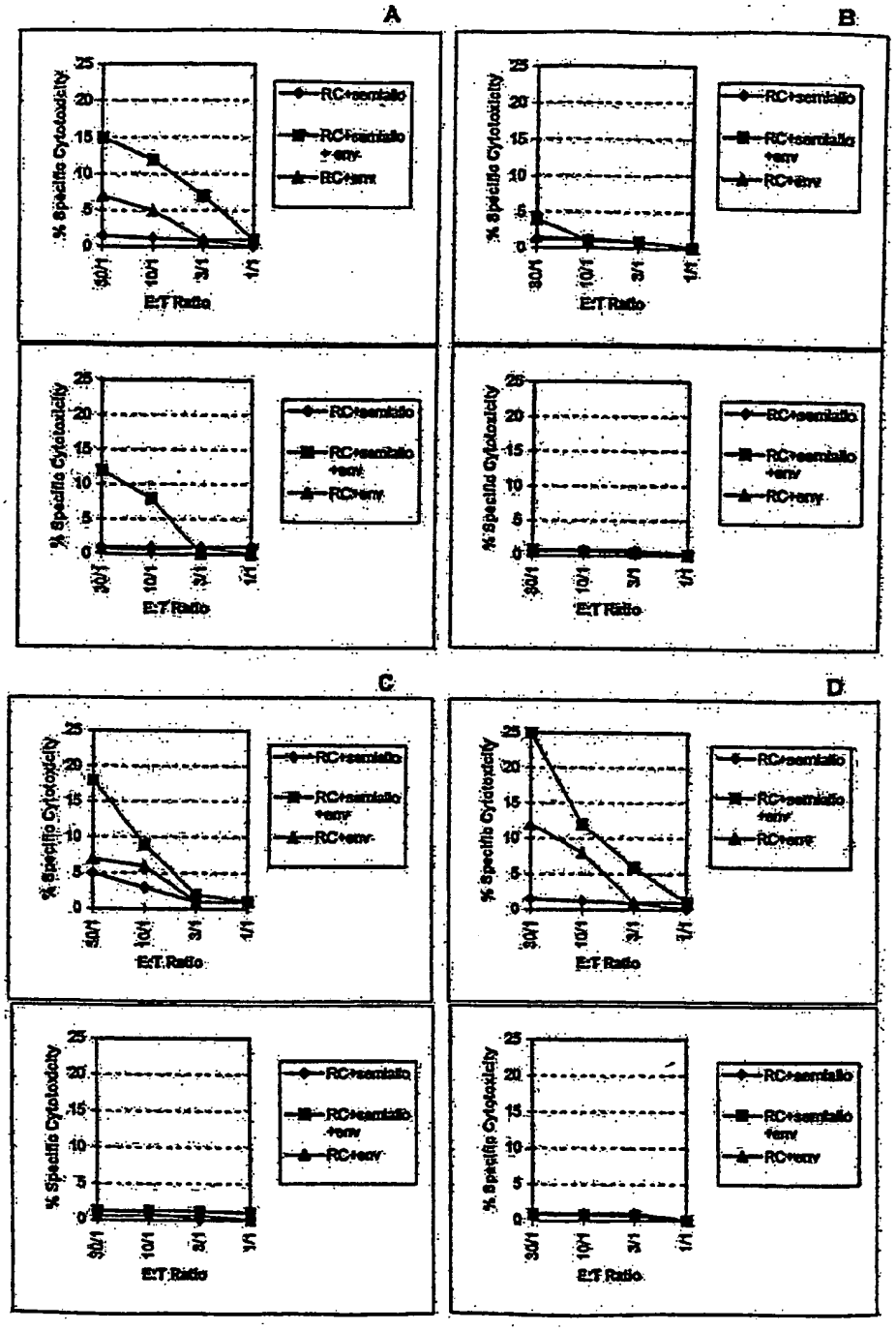

A
B
C
D
% Specific Cytotoxicity
E:T Ratio
30/1
10/1
3/1
1/1
RC+semliki
RC+semliki +env
RC+env

FIG. 9

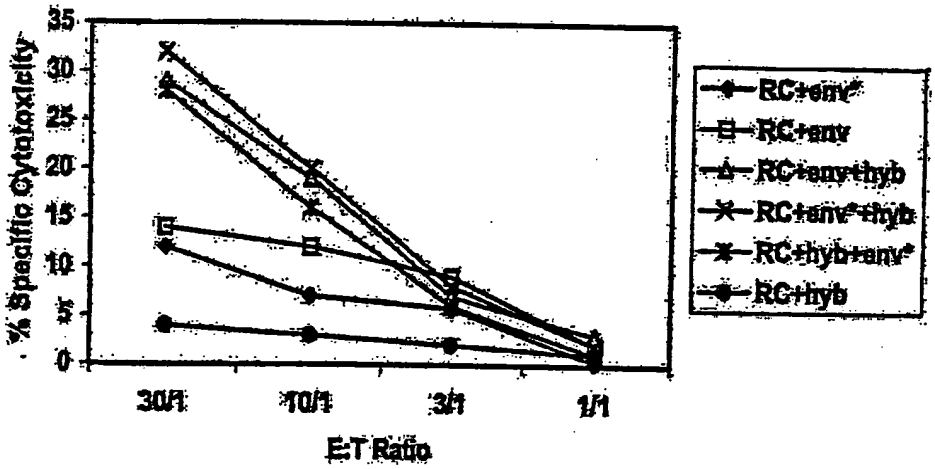

% Specific Cytotoxicity
35
30
25
20
15
10
5
0
30/1
10/1
3/1
1/1
E:T Ratio
RC+env*
RC+env
RC+env+hyb
RC+env*+hyb
RC+hyb+env*
RC+hyb

FIG. 10

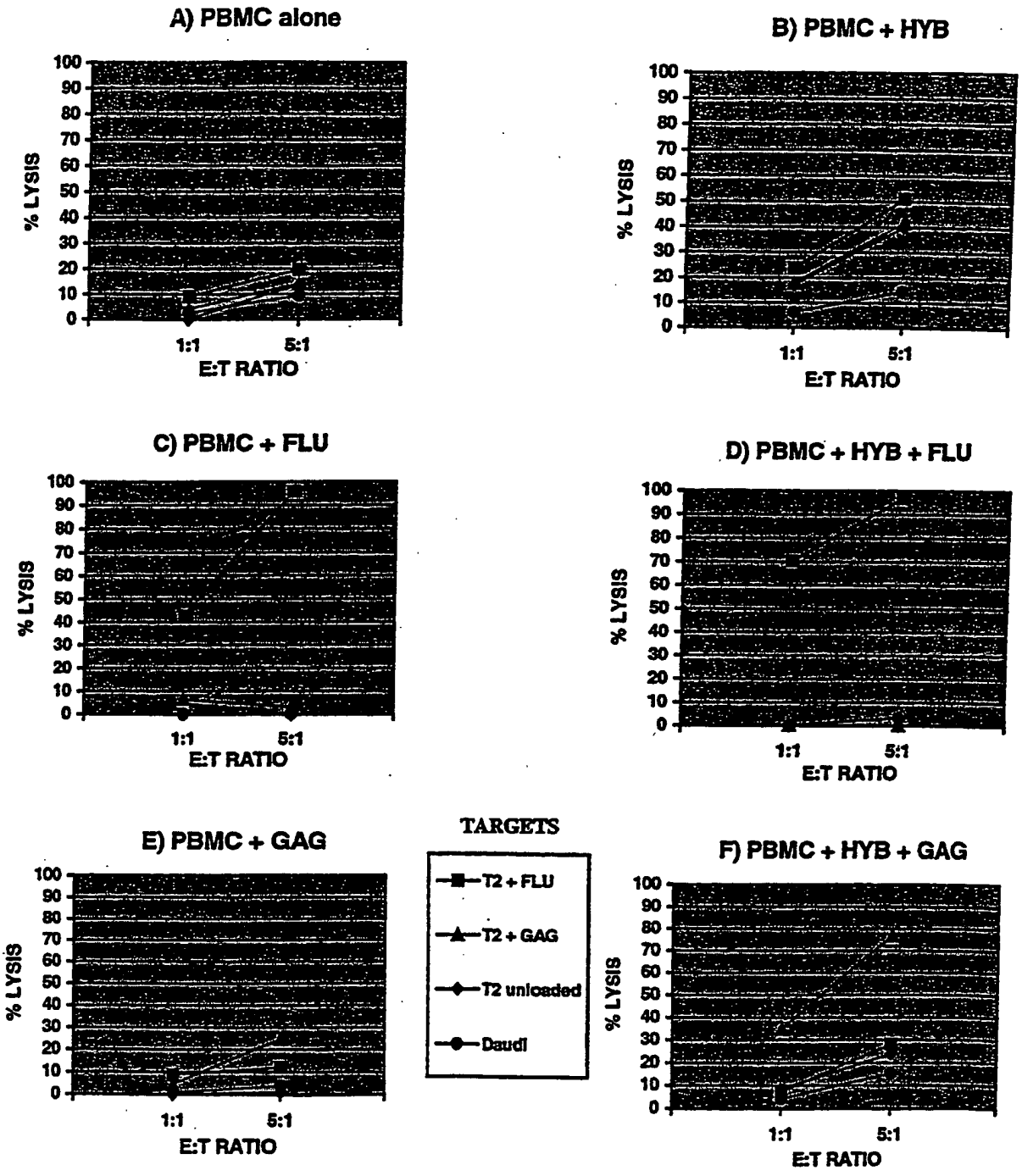

A) PBMC alone
B) PBMC + HYB
C) PBMC + FLU
D) PBMC + HYB + FLU
E) PBMC + GAG
F) PBMC + HYB + GAG
% LYSIS
E:T RATIO
1:1
5:1
0
10
20
30
40
50
60
70
80
90
100
TARGETS
T2 + FLU
T2 + GAG
T2 unloaded
Daudi

FIG. 11

(12) INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(19) World Intellectual Property Organization
International Bureau

(43) International Publication Date
21 December 2000 (21.12.2000)

PCT

(10) International Publication Number
WO 00/76537 A3

(51) International Patent Classification[7]: C12N 5/06, 5/22, A61K 39/21, 39/39, 38/16

(21) International Application Number: PCT/US00/11008

(22) International Filing Date: 24 April 2000 (24.04.2000)

(25) Filing Language: English

(26) Publication Language: English

(30) Priority Data:
09/254,556 16 June 1999 (16.06.1999) US

(63) Related by continuation (CON) or continuation-in-part (CIP) to earlier applications:
US 09/254,556 (CIP)
Filed on 16 June 1999 (16.06.1999)
US PCT/US97/15920 (CIP)
Filed on 10 September 1997 (10.09.1997)
US 09/254,556 (CIP)
Filed on 16 June 1999 (16.06.1999)

(71) Applicants *(for all designated States except US)*: **THE GOVERNMENT OF THE UNITED STATES OF AMERICA, as represented by THE SECRETARY, DEPARTMENT OF HEALTH AND HUMAN SERVICES** [US/US]; National Institutes of Health, Office of Technology Transfer, Suite 325, 6011 Executive Boulevard, Rockville, MD 20852-3804 (US). **MEDICAL UNIVERSITY OF SOUTH CAROLINA** [US/US]; 171 Ashley Avenue, Charleston, SC 29425 (US).

(72) Inventors; and

(75) Inventors/Applicants *(for US only)*: **GATTONI-CELLI, Sebastiano** [US/US]; 1165 Cheresonese Round, Mount Pleasant, SC 29464 (US). **SHEARER, Gene** [US/US]; 5512 Glenwood Road, Bethesda, MD 20817 (US). **GRENE, Edith** [US/US]; 9103 Friars Road, Bethesda, MD 20817 (US). **NEWTON, Danforth, A.** [US/US]; 1673 Dogwood Road, Charleston, SC 29414 (US). **BROWN, Edwin, A.** [US/US]; 773 Creekside Drive, Mount Pleasant, SC 29464 (US). **BERZOFSKY, Jay, A.** [US/US]; 5908 Bradley Boulevard, Bethesda, MD 20814-1107 (US). **DEGROOT, Anne, S.** [US/US]; 292 Morris Avenue, Providence, RI 02906 (US).

(74) Agents: **MILLER, Mary, L.** et al.; Needle & Rosenberg, P.C., Suite 1200, 127 Peachtree Street, N.E., Atlanta, GA 30303 (US).

(81) Designated States *(national)*: AE, AG, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, CA, CH, CN, CR, CU, CZ, DE, DK, DM, DZ, EE, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MA, MD, MG, MK, MN, MW, MX, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TR, TT, TZ, UA, UG, US, UZ, VN, YU, ZA, ZW.

(84) Designated States *(regional)*: ARIPO patent (GH, GM, KE, LS, MW, SD, SL, SZ, TZ, UG, ZW), Eurasian patent (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European patent (AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE), OAPI patent (BF, BJ, CF, CG, CI, CM, GA, GN, GW, ML, MR, NE, SN, TD, TG).

Published:
— *With international search report.*

(88) Date of publication of the international search report:
3 May 2001

For two-letter codes and other abbreviations, refer to the "Guidance Notes on Codes and Abbreviations" appearing at the beginning of each regular issue of the PCT Gazette.

WO 00/76537 A3

(54) Title: USE OF SEMI-ALLOGENEIC CELL LINE-PEPTIDE COMPLEXES FOR THE TREATMENT OF CANCER, AIDS AND OTHER VIRAL DISEASES

(57) Abstract: The present invention provides a composition comprising a semi-allogeneic hybrid fusion cell and an immunogenic peptide. In particular, isolated peptides of HIV (Human Immunodeficiency Virus), HTLV-1, Hepatitis B virus, Hepatitis C virus, rubeola virus, influenza A virus and Human Papilloma Virus are provided in the compositions of the present invention. Moreover, isolated cancer-specific peptides specific to a cancer, for example, B cell lymphoma, T cell lymphoma, myeloma, leukemia, breast cancer, pancreatic cancer, colon cancer, lung cancer, renal cancer, liver cancer, prostate cancer, melanoma and cervical cancer are provided in the compositions of the present invention.

INTERNATIONAL SEARCH REPORT

Interna .al Application No
PCT/US 00/11008

A. CLASSIFICATION OF SUBJECT MATTER
IPC 7 C12N5/06 C12N5/22 A61K39/21 A61K39/39 A61K38/16

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
IPC 7 C12N

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)
EPO-Internal, PAJ, WPI Data, BIOSIS

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category °	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	GRENE E. ET AL.: "Alloimunization for immune based therapy in AIDS malignancies" JOURNAL OF ACQUIRED IMMUNE DEFICIENCY SYNDROMES., vol. 21, 1 May 1999 (1999-05-01), page a16 XP000974801 NEW YORK US abstract --- -/--	1,2, 4-11, 18-21

[X] Further documents are listed in the continuation of box C.

[X] Patent family members are listed in annex.

° Special categories of cited documents :

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier document but published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention .

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.

"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
13 December 2000	27/12/2000

Name and mailing address of the ISA	Authorized officer
European Patent Office, P.B. 5818 Patentlaan 2 NL – 2280 HV Rijswijk Tel. (+31–70) 340–2040, Tx. 31 651 epo nl, Fax (+31–70) 340–3016	De Kok, A

Form PCT/ISA/210 (second sheet) (July 1992)

INTERNATIONAL SEARCH REPORT

Internal Application No
PCT/US 00/11008

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	WO 98 11202 A (UNIV SOUTH CAROLINA (US)) 19 March 1998 (1998-03-19) page 7, line 1 -page 11, line 13 page 17, line 1 - line 25 examples 1- page 15, line 1 - line 13 page 16, line 5 - line 12	1-9,12, 13, 18-23, 25-29, 31-39, 41-45
X	WO 98 33527 A (COHEN EDWARD P) 6 August 1998 (1998-08-06) page 6, last paragraph -page 10, paragraph 2	1,3, 38-45
X	EP 0 273 716 A (USA) 6 July 1988 (1988-07-06) page 3, line 15 -page 8, line 23	14,16,17
A		10,11
X	WO 93 19775 A (MEDIMMUNE INC) 14 October 1993 (1993-10-14) page 3, line 16 - line 35 page 6, line 17 -page 7, line 7	14-17
A		10-13,24
X	HOSMALIN A. ET AL.: "Priming with T helper cell epitopes enhances the antibody response to the envelope glycoprotein of HIV-1 in primates" JOURNAL OF IMMUNOLOGY., vol. 146, 1991, pages 1667-1673, XP000971372 BALTIMORE US ISSN: 0022-1767 abstract	14-17
A		10
A	SHEARER G M ET AL: "Alloimmunization for immune-based therapy and vaccine design against HIV/AIDS" IMMUNOLOGY TODAY, vol. 20, no. 2, February 1999 (1999-02), pages 66-71, XP004157278 CAMBRIDGE GB ISSN: 0167-5699 cited in the application the whole document	1

-/--

1

Form PCT/ISA/210 (continuation of second sheet) (July 1992)

INTERNATIONAL SEARCH REPORT

Interna ial Application No
PCT/US 00/11008

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category °	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	WO 91 09869 A (MEDICAL RES COUNCIL ;UNIV OXFORD (GB)) 11 July 1991 (1991-07-11) page 2, line 4 -page 6, line 22 page 8 -page 9	1,2,8, 12,13, 15,18,22
P,X	NEWTON D.A. ET AL.: "Semiallogeneic cell hybrids as therapeutic vaccines for cancer" JOURNAL OF IMMUNOTHERAPY., vol. 23, March 2000 (2000-03), pages 246-254, XP000971375 NEW YORK US ISSN: 1053-8550 the whole document	1,3-6, 38,39, 41-45

Form PCT/ISA/210 (continuation of second sheet) (July 1992)

INTERNATIONAL SEARCH REPORT

Information on patent family members

Internat .al Application No
PCT/US 00/11008

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
WO 9811202 A	19-03-1998	US 6063375 A	16-05-2000
		AU 4338297 A	02-04-1998
		EP 0927244 A	07-07-1999
WO 9833527 A	06-08-1998	EP 1012240 A	28-06-2000
EP 0273716 A	06-07-1988	AT 92934 T	15-08-1993
		AU 592258 B	04-01-1990
		AU 1365788 A	27-07-1988
		DE 3787002 A	16-09-1993
		DE 3787002 T	25-11-1993
		IL 84980 A	10-06-1993
		JP 7062031 B	05-07-1995
		JP 63503227 T	24-11-1988
		WO 8805051 A	14-07-1988
		US 5939074 A	17-08-1999
		US 5081226 A	14-01-1992
WO 9319775 A	14-10-1993	NONE	
WO 9109869 A	11-07-1991	CA 2072351 A	06-07-1991
		EP 0510054 A	28-10-1992
		GB 2255093 A,B	28-10-1992
		GB 2273709 A,B	29-06-1994
		GB 2273710 A,B	29-06-1994
		US 5700469 A	23-12-1997
		US 5480967 A	02-01-1996